

# Lab recharge 2020

Life science research solutions for pharma



# **Table of contents**

Tools to support your science	. 3
Isolation and sequencing	. 4
Delivering precision health: the role of molecular diagnostics Featured products	
Cell preparation	. 6
Ficoll-Paque PREMIUM density gradient media Featured products	
Cell culture	. 8
Production of recombinant monoclonal antibody (rmAb) in Chinese hamster ovary cells using HyClone CDM4CHO	
medium and HyClone Cell Boost 2 feed supplement	8
Featured products	Q

Sample prep 10
Putting dissolution to the test: importance of filtration
Featured products 11
Save time in HPLC prep 12
Guide to laboratory filtration 13
Purification14
ÄKTA start
Protein purification protocols 14
Swedish scientists make amazing spider
silk from modified <i>E. coli</i> bacteria 16
Featured products17
Analysis 18
DIBE technology for reliable HCP detection
Featured products 19

# How we give back



#### Supporting the Breast Cancer Research Foundation with Think Pink

We did it... Together, with our valued customers, we raised **\$121 653** for the Breast Cancer Research Foundation (BCRF) in 2019. This funds over **2500** hours of revolutionary research into the most common cancer in women worldwide, moving all of us that much closer to a cure.

For more details click **here**.

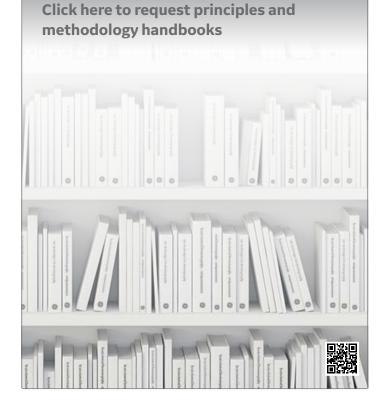




### **People-Planet-Purpose**

We help therapy innovators, researchers, and healthcare providers accelerate how precision diagnostics and therapies are invented, made, and used. Our products enable biological analysis, research, development, and the manufacture of advanced therapies and vaccines. We work with the highest integrity, a compliance culture, and respect for human rights while also reducing the impact of our technology and environmental footprint.

# **Tools to support your science**



Handbooks



# Apps

Click to download our online tools to help you save time in the lab



# Samples and custom quotations

Click here to connect with your Fisher representative and request product information and samples



# Delivering precision health: the role of molecular diagnostics

In recent years there has been an unmistakable trend towards precision health, a broad, all-encompassing approach that uses innovations in technology, diagnostics, and bioinformatics to enhance disease prevention, diagnosis, treatment, and monitoring.

Molecular diagnostics approaches are now instrumental in delivering precision health to patients in many clinical areas, and their role is likely to become even more prominent in years to come.

#### **Revolutionizing healthcare**

The 'All of Us' program was established in 2015. This \$215 million program is an ambitious effort launched by the US government and the National Institutes of Health (NIH) to gather data from one million people "with the ultimate goal of accelerating research and improving health". In this program, "researchers will use data from the program to learn more about how individual differences in lifestyle, environment, and biological makeup can influence health and disease (1)."

The All of Us program fits well within a broader trend of initiatives and collaborations being launched by governments, hospitals, and industry to revolutionize healthcare at a fundamental level. These projects take a multidisciplinary approach to gather, collate, and analyze data for improving population health and wellbeing. Many of them aim to contribute to delivering precision health.

#### What is precision health?

Precision health can be defined as an approach to healthcare that is patientcentric and patient-specific at every step. It applies this personalization to disease prevention, diagnosis, treatment, and monitoring.

Understanding the nuances of an individual's genetics and epigenetics is key to achieving many aspects of precision health. This improved understanding, combined with increasingly easy access to genetic information, has led to a vastly expanded role of genetics in the clinic.

The term 'precision medicine' is frequently used to refer to the use of genetics to help tailor patient therapies. The potential benefits of implementing a precision health approach are wide ranging. Not only can it result in improved survival rates for diseases such as cancer, it can also improve prevention through screening, reduce adverse effects of treatments, and avoid money being wasted on treatments that are ineffective for a given patient.

#### Precision through molecular diagnostics

The genetic information required to deliver precision health can come from a range of clinical diagnostic tests. Advances in molecular in vitro diagnostic assays (IVD assays) have been a key driver for better genetic information, including increased precision, faster turnarounds, and reduced costs.

Assays based on polymerase chain reaction (PCR) currently have the highest market share among the most common molecular diagnostic assays for detecting genetic abnormalities. Other established methods include DNA microarrays and fluorescent in situ hybridization (FISH).

DNA sequencing methods such as Sanger sequencing also have a long track record in finding mutations, but Sanger sequencing has limitations in throughput. Now, the massively parallel sequencing capabilities of next generation sequencing (NGS) approaches have largely replaced Sanger sequencing in many research applications and are making fast inroads into the clinic.

These technologies contribute to precision health by identifying the genetic abnormalities underlying diseases, resulting in diagnoses and treatments based on more than just symptoms. In oncology for example, the genetic makeup of tumors can be highly variable, even when the physical appearance is similar. Characterizing tumors genetically can therefore contribute substantially to personalizing treatments and maximizing their effectiveness. The ways in which clinicians use molecular diagnostics to deliver precision health vary greatly between different clinical areas. For example, decisions on treatment for infectious diseases might require analyzing bacterial or viral genomes, whereas cancer diagnostics focus on genome markers in germline or tumor DNA. The following sections provide an overview of these two clinical areas, describing how molecular IVD tests contribute to precision health, giving examples of current approaches, and outlining future perspectives.

#### **Precision health in cancer**

High-precision approaches are well suited to personalized cancer therapy due to high variability, not just in the causes of cancer, but also in treatments and their side effects. Molecular diagnostics already play a substantial role in oncology, one which in the future is likely to increase at a rapid pace.

Worldwide, an estimated 18.1 million people are diagnosed with cancer every year, leading to 9.6 million deaths. The most common types are lung, breast, and colorectal cancer. For each of these cancers, molecular assays are widely used to enable reliable screening or to guide clinicians in making critical decisions for personalized cancer treatment (2).

#### References

- 1. All of Us Research Program, World Health Organization *allofus.nih.gov.* Accessed 7 May 2019.
- Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018, The International Agency for Research on Cancer (IARC) who.int/cancer/PRGlobocanFinal.pdf. Accessed 7 May 2019.

# Sera-Mag<sup>™</sup> SpeedBeads and Sera-Mag Streptavidin-coated magnetic particles

Provide a high biotin-binding capacity along with a strong affinity for targeted, biotin labeled molecules. Available with low (2500 to 3500 pmol/mg), medium (3500 to 4500 pmol/mg) or high (4500 to 5500 pmol/mg) nominal biotin binding capacities for optimizing assay development.

### Sera-Mag SpeedBeads and Sera-Mag Carboxylate-Modified Magnetic Particles

Combine a fast magnetic response time and high binding capacity with a large surface area, high sensitivity, stability, physical integrity, and fast reaction kinetics. Typical applications include sample preparation, proteomics, nucleic acid isolation, and immunoassay applications. Carboxylic groups on the surface permit easy covalent coupling to biomolecules of interest using convenient carbodiimide chemistry.

## **Nucleon BACC Genomic DNA Extraction Kits**

Designed for rapid extraction of high-quality, high molecular weight genomic DNA from blood and cell cultures. The Nucleon proprietary resin is added following cell lysis, deproteinization with sodium perchlorate, and a single chloroform extraction.

- Size of recovered DNA ranges from 23 to 250 kbp.
- Features a phenol-free protocol requiring only 30 min to complete.
- Nucleon HT format enables DNA extraction from hard tissues & paraffin sections



#### Webinar: Introducing Lyo-Stable Technology

Our lyophilization services enable sample integrity and stability to be maintained at a wider range of temperatures for up to two years, helping to improve results and data reliability. Watch webinar **here**.



Chemistry	Format	Description	Volume	Pack size	ltem
Nucleon resin	Kit	Nucleon BACC1	25 preps	1/pk	RPN8501
Nucleon resin	Kit	Nucleon HT	50 preps	1/pk	RPN8509
Nucleon resin	Kit	Nucleon BACC3	50 preps	1/pk	RPN8512
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBeads Streptavidin-Blocked	100 mL	1/pk	21152104010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBeads Streptavidin-Blocked	1 mL	1/pk	21152104011150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Carboxylate-Modified Magnetic Particles (Hydrophylic)	100 mL	1/pk	24152105050350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 2500 to 3500 (low) pmol per mg	5 mL	1/pk	30152103010150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 2500 to 3500 (low) pmol per mg	100 mL	1/pk	30152103010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 3500 to 4500 (med) pmol per mg	5 mL	1/pk	30152104010150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 3500 to 4500 (med) pmol per mg	100 mL	1/pk	30152104010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 4500 to 5500 (high) pmol per mg	5 mL	1/pk	30152105010150
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Streptavidin-Coated - 4500 to 5500 (high) pmol per mg	100 mL	1/pk	30152105010350
Sera-Mag Magnetic Beads	Bottle	Sera-Mag Carboxylate-Modified Magnetic Particles (Hydrophobic)	15 mL	1/pk	44152105050250
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBead Carboxylate (Hydrophylic)	15 mL	1/pk	45152105050250
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBead Carboxylate (Hydrophobic)	15 mL	1/pk	65152105050250
Sera-Mag Magnetic Beads	Bottle	Sera-Mag SpeedBead Carboxylate (Hydrophobic)	100 mL	1/pk	65152105050350
Silica coated beads	Kit	SeraSil-Mag 400 <b>NEW</b>	5 mL	1/pk	29357369
Silica coated beads	Kit	SeraSil-Mag 400 <b>NEW</b>	60 mL	1/pk	29357371
Silica coated beads	Kit	SeraSil-Mag 700 <b>NEW</b>	5 mL	1/pk	29357373
Silica coated beads	Kit	SeraSil-Mag 700 <b>NEW</b>	60 mL	1/pk	29357374









# Ficoll-Paque<sup>™</sup> PREMIUM density gradient media

Ficoll-Paque PREMIUM products are a range of sterile, ready-to-use density gradient media for the preparation of mononuclear cells. All Ficoll-Paque PREMIUM products have low endotoxin levels (< 0.12 EU/mL) and are manufactured under a Quality Management System certified to ISO 13485 and to the guidelines outlined in EU GMP Annex 1: Manufacture of Sterile Medicinal Products (1). Ficoll-Paque PREMIUM products are available in densities of 1.073, 1.077, and 1.084 g/mL for the preparation of different density preparations of mononuclear cells from peripheral blood, bone marrow, umbilical cord blood, and placental tissue. Mononuclear cell isolation can be automated and functionally closed by using Sepax<sup>™</sup> technology (2, 3).

#### **Features**

- Manufactured within a quality management system certified to ISO 13485.
- Meet USP <1043> 'ancillary materials for cell, gene, and tissue engineered products', within the responsibilities applicable to a supplier (4).
- Suitable for in vitro applications.
- Sterile, ready-to-use reagent.
- Low levels of endotoxin (< 0.12 EU/mL) secured and tested.

Classical Ficoll-Pague PREMIUM with a density of 1.077 g/mL was developed from Ficoll-Pague PLUS, which is based on Ficoll™ PM400 (polysucrose) and sodium diatrizoate and has a more than 40 yr track record for large- or smallscale purification of mononuclear cells from human peripheral blood. All Ficoll-Paque PREMIUM products differ from Ficoll-Paque PLUS in that they are manufactured under a Quality Management System certified to ISO 13485 and to the guidelines outlined in EU GMP Annex 1: Manufacture of Sterile Medicinal Products (3). These require stringency in validation and documentation of manufacturing procedures.



### Applications Ficoll-Paque PREMIUM

Ficoll-Paque PREMIUM has a density of 1.077 g/mL and is optimized for the isolation of mononuclear cells from human peripheral blood by using a simple and rapid centrifugation technique developed by Bøyum et al. (5). The medium can also be used for the isolation of human mononuclear cells from other sources, including bone marrow and umbilical cord blood. Separation of normal human peripheral blood by the recommended protocol typically yields a mononuclear cell preparation with:

- 95% ± 5% mononuclear cells present in the separated fraction
- > 90% viability of the separated cells
- 60% ± 20% recovery of the mononuclear cells present in the original blood sample
- 3% ± 2% granulocytes
- 5% ± 2% red blood cells



Save time in the lab by using our Percoll™ Calculator

Click *here* to use it.





### **Ficoll-Paque PLUS and Ficoll-Paque PREMIUM**

Table comparing the different Ficoll products.

Parameter	Ficoll-Paque PLUS	Ficoll-Paque PREMIUM	Ficoll-Paque PREMIUM 1.073	Ficoll-Paque PREMIUM 1.084			
Application	Isolation of human mononuclear cells for <i>in vitro</i> studies. For research use only	Isolation of mononuclear cells from human peripheral blood, bone marrow, and umbilical cord blood		Isolation of a broad range of human mononuclear cells including those of a higher density and for separating blood cells from mice or rats			
Density	1.077 g/mL	1.077 g/mL	1.073 g/mL	1.084 g/mL			
Osmolality	-	288 to 310 mOsm/kg	276 to 298 mOsm/kg	322 to 344 mOsm/kg			
Regulatory	-	Manufactured under a Quality	Management System certified t	o ISO 13485			
Physical state			Liquid				
Endotoxin activity max.		< 0.	12 EU/mL				
pH range		5	.5 to 7.5				
Color		Colorless	to slight yellow				
Sterility	ŀ	Autoclave steam sterilization with sterility assurance level (SAL) of 10 <sup>-6</sup>					
Estimated shelf life/ Stability	At least 3 yr from manufacture date under recommended storage conditions. Deterioration of Ficoll-Paque products is indicated by the appearance of a yellow color or particulate material in the solution						
Storage conditions		4°C to 30°C and	d protected from light				

### **Percoll and Percoll PLUS**

Are silica-based colloidal media for cell separation by density gradient centrifugation

#### **Percoll offers:**

- Low osmolality: can easily be adjusted with physiological saline, cell culture medium, or sucrose to give gradients that are iso-osmotic throughout
- Low viscosity resulting in rapid formation of gradients and particle separation at low centrifugal forces
- Support through extensive research use: Thousands of publications on Percoll in scientific journals
- Formation of either continuous preformed or self-generated gradients by centrifugation at moderate speeds

#### **Percoll PLUS offers:**

- Low endotoxin levels (max. 2 EU/mL)
- · Absence of toxicity for cells and very low chemical reactivity
- Low osmolality: can easily be adjusted with physiological saline, other balanced salt solutions, or cell culture media, to give gradients that are iso-osmotic throughout
- Low viscosity resulting in rapid formation of gradients and particle separation at low centrifugal forces

Product type	Format	Description	Pack size	Item
Sucrose Polymer	Bag	Ficoll PM400	5 kg	17030005
Sucrose Polymer	Bag	Ficoll PM400	500g	17030050
Media	Bottle	Percoll	1 L	17089101
Media	Bottle	Percoll	250 mL	17089102
Media	Bottle	Ficoll-Paque PLUS	6 × 100 mL	17144002
Media	Bottle	Ficoll-Paque PLUS	6 × 500 mL	17144003
Media	Bottle	Percoll PLUS	1 L	17544501
Media	Bottle	Percoll PLUS	250 mL	17544502
Media	Bottle	Ficoll-paque PREMIUM 1.084	6 × 100 mL	17544602
Media	Bottle	Ficoll-paque PREMIUM 1.073	6 × 100 mL	17544652





# Production of recombinant monoclonal antibody (*r*mAb) in Chinese hamster ovary cells using HyClone<sup>™</sup> CDM4CHO medium and HyClone Cell Boost<sup>™</sup> 2 feed supplement

One of the most important cell lines used in the production of recombinant proteins is the Chinese hamster ovary (CHO) cell line. Regulatory concerns surrounding the use of animal-derived components in the production of therapeutic proteins is a major driver for the development of chemically defined and animal-derived component-free (ADCF) media for CHO cell growth and protein production. This application note demonstrates the performance of the chemically defined HyClone CDM4CHO base medium optimized for CHO cells. To increase process yield, the CHO cell culture was supplemented with HyClone Cell Boost 2.

#### Introduction

HyClone CDM4CHO is a CHO cell culture medium free of protein and animalderived components. This regulatoryfriendly medium is developed to increase process yields for the industrial manufacture of recombinant proteins using a variety of CHO cell clones. This medium has been successfully tested in a variety of culture systems, including T-flasks, shaker flasks, and bioreactors including fed-batch and perfusion culturing. In this study, an *r*mAb-producing CHO cell clone was cultured in a stirredtank bioreactor.

To optimize process yields with HyClone CDM4CHO medium, the culture was fed HyClone Cell Boost 2 supplement. This supplement is designed to provide nutrients such as carbohydrates, amino acids, and vitamins as part of a fedbatch culture strategy, and has been developed for recombinant protein production with various cell lines including CHO cells.

#### **Materials and methods**

An rmAb-producing CHO cell line was used in this study. Cells were grown in 1.5 L suspension cultures in HyClone CDM4CHO medium using a 3 L stirredtank bioreactor (Applicon). Culture temperature was controlled at 37°C, dissolved oxygen was controlled at 50% air saturation, and culture pH at 7.0. The culture was pulse-fed at 40 mL/L with HyClone Cell Boost 2 supplement (hydrated at 149 g/L in injection-grade water and pH adjusted to  $\ge$  9.5) on day 4 to 8.

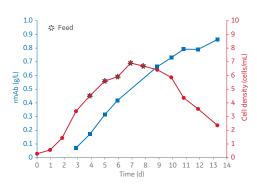
#### Results

Viable cell density reached a maximum of  $6.9 \times 10$  cells/mL within the culture span of 13.5 days (Fig 1). The *r*mAb yield

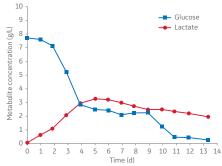
for the process was 0.86 g/L. Glucose was added to the culture as needed when fed with HyClone Cell Boost 2 supplement. Glucose and lactate profiles are shown (Fig 2). As shown, cells started to metabolize lactic acid after day 5. Using the described fedbatch process, productivity was improved 2–4-fold compared with initial production levels (0.1796 g/L) in batch mode (Fig 3).

#### Conclusion

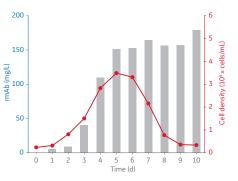
CHO cells were successfully grown in fed-batch suspension culture utilizing HyClone CDM4CHO medium supplemented with HyClone Cell Boost 2. Compared with initial production levels in batch mode, a significant productivity improvement could be achieved with the described fed-batch process.



**Fig 1.** Cell density and *r*mAb production in fed-batch CHO cell culture using HyClone CDM4CHO base medium and HyClone Cell Boost 2 feed supplement.



**Fig 2.** Glucose and lactate profiles of CHO cells in fed-batch cultures using HyClone CDM4CHO base medium and HyClone Cell Boost 2 feed supplement.



**Fig 3.** Cell density and *r*mAb production in batch CHO cell culture using HyClone CDM4CHO base medium.

### HyClone Fetal Bovine Serum FBS from origins USA, Australia and New Zealand

HyClone Defined Fetal Bovine Serum (FBS) is our highest quality FBS and is widely used by cell culturists who have a concern for viral contaminants and require an extensive biochemical profile.

HyClone defined FBS is filtered through serial 40 nm pore size-rated filters, which are the most retentive filters used in commercial FBS production and has over 50 components analyzed on the finished product and the results are included in the certificate of analysis and the biochemical assay list.

### **HyClone Cell Boost supplements**

HyClone Cell Boost supplements each provide an exclusive selection of nutrients such as amino acids, vitamins, lipids, cholesterol, glucose and/or growth factors in complements optimized for multiple mammalian cell types.

These supplements are chemically defined and contain no animal derived components and are designed to increase cell productivity in a variety of cell lines.

### HyClone WFI quality water to either US or EU specifications

Water for injection (WFI) quality water is widely used in the pharmaceutical industry.

- Meets stringent Pharmacopeial specifications.
- Manufactured in an ISO 9001 certified facility.
- Certificate of analysis (CoA) for each lot of water produced.
- · Low levels of endotoxin.
- Sterile filtered.







For comprehensive upstream cell culture and bioprocessing toolbox for pharmaceutical research, visit GE Knowledge centre *here.* 



Product type	Format	Description	Volume	Pack size	ltem
Serum	Bottle	US Characterized FBS	500 mL	1/pk	SH30071.03
Serum	Bottle	AUS Characterized FBS	1000 mL	1/pk	SH30084.04
Serum	Bottle	NZ Characterized FBS	1000 mL	1/pk	SH30406.03
Supplement	Bottle	Cell Boost 1 liquid	500 mL	1/pk	SH31113.01
Supplement	Bottle	Cell Boost 2 liquid	500 mL	1/pk	SH31114.01
Supplement	Bottle	Cell Boost 3 liquid	500 mL	1/pk	SH31115.01
Supplement	Bottle	Cell Boost 4 liquid	500 mL	1/pk	SH31116.01
Supplement	Bottle	Cell Boost 5 liquid	500 mL	1/pk	SH31117.01
Supplement	Bottle	Cell Boost 6 liquid	500 mL	1/pk	SH31118.01
Supplement	Bottle	Cell Boost 7a liquid	500 mL	1/pk	SH31119.01
Supplement	Bottle	Cell Boost 7b liquid	100 mL	1/pk	SH31120.01
WFI Water	Bottle	LM-WFI Quality Water-ADCF EU spec	20L	1/pk	CH30154.02
WFI Water	Bottle	LM-WFI Quality Water-ADCF EU spec	1L sample	1/pk	CH30154.07
WFI Water	Bottle	LM-WFI Quality Water-ADCF EU spec	1L	1/pk	CH30154.08
WFI Water	Bottle	LM-WFI Quality Water-ADCF EU spec	5L	1/pk	CH30154.10
WFI Water	Bottle	LM-WFI Quality Water-ADCF EU spec	20L	1/pk	CH30154.11



# Putting dissolution to the test: importance of filtration

Dissolution testing provides critical drug release information on solid dosage forms, essential for pharma QC and drug development Read about filtration's key role in the process to improve throughput and reduce result variability.

#### What is dissolution testing?

Drug dissolution testing is a routine test used in the pharmaceutical industry to provide critical in vitro drug release profiles, evaluating the rate of release of an active pharmaceutical ingredient (API) from its dosage form.

This information is essential for pharmaceutical quality control (pharma QC) in assessing batch-to-batch consistency of solid dosage forms, such as tablets, pills and capsules. It is also useful in drug development for predicting in vivo behavior and release profiles of different formulations.

The test involves several key steps:

- Dissolution of a solid dosage form under controlled conditions in a dissolution vessel.
- 2. Collection at specific time points.
- 3. Sample preparation, including filtration.
- Analysis of each sample to determine the amount of drug dissolved at certain time points. Analysis tools include high-performance liquid chromatography (HPLC) and UV-vis spectrophotometry.

We talk about *automated dissolution testing methods* in a recent article from our knowledge center. In this blog, I'll focus on the sample preparation stage and the importance of the filtration step for producing accurate and reproducible results.

# Why is filtration important in dissolution testing?

The aim of any drug dissolution test is to determine the amount of API dissolved at chosen time points as samples are withdrawn from the dissolution vessel. Filtration is key to this process as it stops the dissolution process, effectively freezing the sample state and making sure it accurately represents a single and specific time point during dissolution. Filtration separates the dissolved drug from the undissolved dosage components, enabling sample analysis that determines the amount of drug dissolved.

This downstream analysis is often carried out through HPLC or UV-vis spectrophotometry, so good sample preparation, including efficient filtration, is necessary for accurate results. Impurities can affect the analysis and might damage the HPLC column.

Despite the importance of the sample preparation stage, it's often overlooked during method development and throughout dissolution testing. As a result, laboratories might not be using the most appropriate membrane filters for the job, and this could be influencing results.

# What should I consider when choosing a filter for dissolution testing?

It's often the case that the syringe filter you routinely use for dissolution testing is just the one that's available in-house. But, evaluating potential filter membranes during method validation can help researchers optimize the technique before transferring the dissolution test process to the QC lab. This optimization can also help maximize result accuracy and reproducibility.

There are three key filter characteristics to consider when evaluating membranes:

• Does the membrane have a broad chemical compatibility, suitable for use with a variety of solvents and APIs?

- Is a good drug recovery possible because of low analyte/drug binding to the membrane?
- Does the membrane show a low level of extractables, to avoid introducing impurities into the sample?

For all HPLC analyses, chemical compatibility and low levels of extractables are important considerations for reducing the risk of new contaminants interfering with results, and determining that the membrane filter, the solvents and APIs being investigated are well-matched.

Hydrophilic PTFE membranes have broad chemical compatibility, and so are not likely to introduce extractable impurities into samples. They are also inert and have low levels of analyte binding, which helps maximize recovery from the membrane and avoid inaccuracies in API quantitation.

It's also possible to use *nylon membranes* for dissolution testing, but they tend to have strong drug binding characteristics that might lead to inaccurate API quantitation. However, you can reduce the level of impurities and alleviate some of the drug binding by prerinsing the syringe filter. It's possible to minimize impurities in both nylon and PTFE syringe filters this way.

The membrane choice you make can affect the success and accuracy of your dissolution testing procedures during drug development and quality control.



### **Roby automated syringe filters**

Roby 25 syringe filters for robotic systems were developed specifically for automated sample filtration and are available with various membranes. For difficult-to-filter samples, Roby syringe filters are also available with membranes plus an integral glass fiber prefilter.

The filter housing is made from mechanically stable polypropylene. The external geometry of the filter housing ensures simple and smooth filter transport from the storage turntable to the filtration site and easy filter changing.

Features and benefits

- · Optimized for automatic dissolution test systems
- Mechanically stable polypropylene
- Easy filter changing
- Ensures simple and smooth filter transport

The Roby filter validation kit offers six different Roby automated syringe filters, along with a filter validation protocol and filter selection aid. Tubes of 25 filters are ready for loading into tablet tester systems.

### 850-DS 8-channel filter plate

Whatman<sup>™</sup> filter plates for use in Agilent<sup>™</sup> 850-DS Dissolution Sampling Station.

Automated processing of up to 8 samples simultaneously. The filter plates are specially designed for Agilent equipment and increase productivity by allowing reliable alignment of the liquid path and reducing the risk of jamming or leaks that may occur with other dissolution sample preparation systems.

Try our **Whatman Filter Selector Tool** to find out if you are using the most appropriate filtration solution for your samples. Click *here* to get there.

Membrane	Format	Description	Format/pore size	Pack size	ltem
RC	Non sterile	Protein Prep syringe filter for ÄKTA™ systems	30 mm 0.2 µm	150/pk	10463043
RC	Non sterile	Protein Prep syringe filter for ÄKTA systems	13 mm 0.45 µm	150/pk	10463113
Absorbent	Sheets	Benchkote™ surface protector for ÄKTA start	310 mm x 210 mm	25/pk	2300-10064
Absorbent	Sheets	Benchkote surface protector	460 mm x 570 mm	50/pk	2300-916
GF92	Non sterile	Roby automated filtration syringe filter	25 mm 1 µm	1000/pk	10463800
RC	Non sterile	Roby automated filtration syringe filter	25 mm 0.45 μm	1000/pk	10463806
Various (6)	Non sterile	Roby automated filter validation kit	25 mm	6x 25/pk	10463898
Nylon	Non sterile	850-DS 8-Channel filter plate	0.45 µm	50/pk	7707-3100
PES	Non sterile	850-DS 8-Channel filter plate	0.2 µm	50/pk	7707-3600
RC	Non sterile	Whatman GD/X™ syringe filter	25 mm 0.2 µm	150/pk	6887-2502
PVDF	Non sterile	Whatman GD/X syringe filter	25 mm 0.45 µm	150/pk	6872-2504
PTFE	Non sterile	Mini-UniPrep™ syringeless filter	0.45 µm	100/pk	UN203NPUORG
RC	Non sterile	Mini-UniPrep syringeless filter	0.45 µm	100/pk	UN203NPURC
PVDF	-	Mini-UniPrep G2 amber syringeless filter	0.2 µm	100/pk	GN203APEAQU
H-PTFE	Non sterile	Puradisc syringe filter <b>NEW</b>	25mm 0.45 µm	200/pk	6773-2504
H-PTFE	Non sterile	Puradisc syringe filter <b>NEW</b>	13mm 0.2 µm	100/pk	6772-1302









# Save time in HPLC prep

Sample filtration protects your HPLC instrument and column while preserving data quality. Read our tips on using multilayer and all-in-one filter units to save time and improve lab efficiency.

If you analyze large numbers of samples using high-performance liquid chromatography (HPLC), sample preparation can take up a lot of your time. Filtering samples before HPLC can help avoid frit clogging while maintaining data quality.

So, what can you do to simplify and speed up the process? Read on to find out!

#### Try a stacked syringe filter

Syringe filtration often involves aspirating the sample, fitting a particle filter, filtering into an autosampler vial, capping, and finally transferring the vial to an autosampler. You might repeat this process dozens of time a day, depending on your circumstances.

If you have difficult-to-filter samples, you might find that high particulate samples can take more time to filter. To help with this, stacked filter devices have multiple layers of filtration, starting with larger pore sizes and going down to the desired pore size.

This approach traps large particles first, and successively traps smaller particles.

The device does not get clogged as easily as devices with a single membrane, making filtration faster and easier.

#### **Go syringeless**

If your samples are reasonably easy to filter, a syringeless filter option simplifies the process greatly.

Using a standard syringe filter involves at least four individual components, five if you include the initial sample storage vial. When you have dozens (or hundreds!) of samples to filter, the multi-step workflow is time consuming and can lead to sample loss.

In a syringeless filter, the filter membrane, pre-filtration chamber, post-filtration storage vial, and cap are all part of one device. This design streamlines HPLC sample prep and minimizes the number of consumables. Filtration can be performed 3 times faster than with syringe filters.

Using a syringeless filter means that you only need to add the sample to the outer chamber, place the plunger, and push. The inner storage vial holds your filtered sample ready for analysis, so it can go directly into your autosampler. Construction can be either polypropylene or glass and the vial can be either clear or amber colored depending on the requirements around your sample.

#### Broaden your solvent compatibility

When your lab prepares a wide variety of sample types using different solvents for HPLC analysis, identifying appropriate membrane materials can be timeconsuming. Different materials might be more or less suitable for a given sample based on chemical compatibility and solvent resistance.

If you want to make filtration easier, you could try out a material with broad solvent compatibility. Regenerated cellulose (RC), for example, is well suited for both hydrophilic and hydrophobic solvents. Using RC for most or even all your samples can reduce time spent researching and selecting materials.

#### Use tools to boost throughput

A multi-compressor can save time when using syringeless units. Filtering multiple samples simultaneously with a dedicated tool can also reduce hand strain.



Whatman GD/X stacked syringe filter

Mini-UniPrep syringeless filters

# **Guide to laboratory filtration**

### Filtration devices for small volume sample preparation

Select the optimal Whatman filter for your application





New filter media available soon for Puradisc and Whatman Uniflo: hydrophilic-PTFE (H-PTFE) H-PTFE membrane can be used for both aqueous and aggressive organic solvents. This membrane is suitable for HPLC/UHPLC sample preparation as well as many other applications in a busy, high volume lab as its dual capability handles most solvents.

# ÄKTA start

## An easy-to-learn and easy-to-use system to remove the hassles of manual protein purification

Purify tagged proteins and antibodies easily. Gain insight from real-time monitoring. Evaluate and share your results.

**User friendly**—Easy-to-use touchscreen display allows you to start the run at the touch of a button

**Convenient**—Easy transition from manual to automatic purification

**Gain deeper insights**—Gain valuable insights from real-time monitoring and control software

**Simplify your workflow**—Purify tagged proteins and antibodies easily using prepacked column

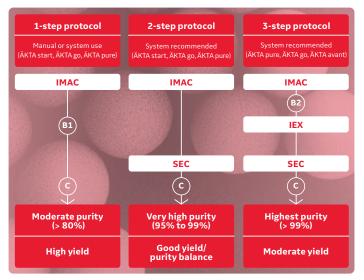
Request information here.



# **Protein purification protocols**

## Histagged protein purification protocol

Purifying histidine (his)-tagged proteins may sound easy. However, there are tips to ensure that you get the most from your his-tagged protein purification protocol, by choosing the right combination of chromatography techniques in a multistep approach. Below are examples for best practice.



IEX = ion exchange chromatography; IMAC = immobilized metal ion affinity chromatography; SEC = size exclusion chromatography; B1 = buffer exchange to remove imidazole or salts; B2 = buffer exchange to prepare for IEX; C = concentration for sample volume reduction, which may also be performed before SEC. Steps in circles are optional and are applied if necessary.

# Which chromatography columns are recommended for each protein purification step?

	1-step protocol	2-step protocol	3-step protocol
IMAC	HisTrap™ HP HisTrap FF crude HisTrap excel HiTrap TALON™ crude	HisTrap HP HisTrap FF crude HisTrap excel HiTrap TALON crude	HisTrap HP HisTrap FF crude HisTrap excel HiTrap TALON crude
IEX			HiTrap™ Q HP HiTrap SP HP HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes
SEC		Superdex 75 Increase HiLoad™ Superdex 75 pg HiPrep™ Sephacryl™ S-100 HR HiPrep Sephacryl S-200 HR	Superdex 75 Increase HiLoad Superdex 75 pg HiPrep Sephacryl S-100 HR HiPrep Sephacryl S-200 HR



Learn more about protein purification protocols in our **Strategies for Protein Purification handbook**. Download handbook *here*.

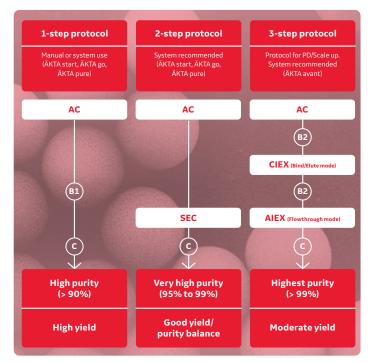


#### **REQUEST INFORMATION**

11

### **Antibody purification protocols**

Antibody purification requires the right balance between purity and yield. Typically they are challenged by two factors: (A) Capturing as many antibodies as possible and without degrading the sample and (B) removing the remaining impurities and minimizing aggregate content.



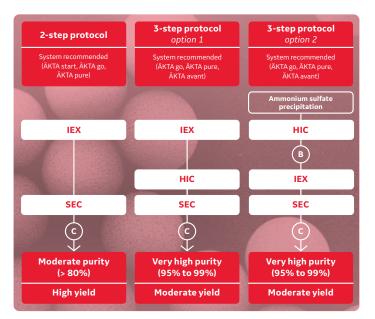
# Which chromatography columns are recommended for each step?

	1-step protocol	2-step protocol	3-step protocol
Affinity	HiTrap Protein A HP HiTrap Protein G HP HiTrap MabSelect™ PrismA HiTrap MabSelect SuRe™	HiTrap Protein A HP HiTrap Protein G HP HiTrap MabSelect PrismA HiTrap MabSelect SuRe	HiTrap Protein A HP HiTrap Protein G HP HiTrap MabSelect PrismA HiTrap MabSelect SuRe
CIEX			HiTrap Capto S ImpAct HiScreen Capto S ImpAct
AIEX			HiTrap Capto Q HiScreen™ Capto Q
SEC		Superdex 200 Increase HiLoad Superdex 200 pg HiPrep Sephacryl S-300 HR	

B1: Buffer exchange to neutralize low pH Ab elution buffer. B2: Buffer exchange to prepare for IEX. C: Concentration for sample volume reduction. (May also be performed before SEC.)

# **Untagged protein purification**

Most proteins purified in laboratory scale are affinity tagged and can therefore be purified with relative ease using affinity chromatography (AC). Sometimes the protein to be purified is untagged for the following reasons: (A) it comes from a natural source (native protein) or (B) the untagged protein is a recombinant protein that has been overexpressed without a tag, which would otherwise interfere with the protein structure or activity. Several reliable approaches to purification of untagged proteins are available.



B: Buffer exchange to prepare for IEX. C: Concentration for sample volume reduction. May also be performed before SEC.

# Which chromatography columns are recommended for each step?

	1-step protocol	2-step protocol	3-step protocol
IEX or HIC	HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes HiTrap Q HP HiTrap SP HP	HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes HiTrap Q HP HiTrap SP HP	HiTrap Phenyl HP HiTrap Phenyl FF HiTrap HIC Selection Kit
HIC or IEX		HiTrap Phenyl HP HiTrap Phenyl FF HiTrap HIC Selection Kit	HiTrap Capto Q ImpRes HiTrap Capto SP ImpRes HiTrap Q HP HiTrap SP HP
SEC	HiLoad Superdex 30 pg HiLoad Superdex 75 pg HiLoad Superdex 200 pg HiLoad Superose 6 pg HiScale SEC columns (on demand)	HiLoad Superdex 30 pg HiLoad Superdex 75 pg HiLoad Superdex 200 pg HiLoad Superose 6 pg HiScale SEC columns (on demand)	HiLoad Superdex 30 pg HiLoad Superdex 75 pg HiLoad Superdex 200 pg HiLoad Superose 6 pg HiScale SEC columns (on demand)

# Swedish scientists make amazing spider silk from modified *E. coli* bacteria

The Stockholm-based biomaterials company is using genetically engineered bacteria and our protein purification technology to produce large quantities of the so-called spidroin proteins found in dragline silk, and then customize them for a variety of specific purposes. "Man-made spider silk can be adjusted to contain specific parts that bind to cells and promote wound healing, thereby enabling use within fields of tissue engineering, diagnostics and cell culture," says Kristina Martinell, Spiber's production director. "In short, it's a tailor-made biomaterial."

Spiber can now manufacture spider silk fiber, film, foam and even mesh. The company says that the material is as strong as mammalian tendons and remains stable at boiling temperatures of up to 267 degrees Celsius (512 Fahrenheit). Over time, the company's technique has evolved to keep the material soluble until it is ready to be shaped into the arrangements needed for various applications.

As a result, the range of potential products is huge. The company is working to apply spider silk in several medical fields, including cardiology, heart tissue regeneration, bone reconstruction, skin cell growth and vaccines.

Read more **here**.





Image credit: Spiber Technologies



# Sign up for the ÄKTA club newsletter for more insights in protein purification



# Capto<sup>™</sup> HiRes – When the highest resolution in IEX matters!

In many research areas, for example in structural biology using X-ray crystallography or cryo-electron microscopy (cryo-EM), obtaining homogeneous size and charge of biomolecules is crucial for the elucidation of their structures. High-resolution separation of samples based on their charge properties is essential to secure sample charge homogeneity and success of the study.

#### Capto Q HiRes and Capto S HiRes replace MonoBeads columns

A separation that worked on a Mono Q or Mono S column may be performed on a Capto HiRes Q or Capto HiRes S column with little modification or optimization. Similar resin selectivity and slightly improved resolution can be expected with the Capto HiRes columns while using the same experimental conditions. The similar selectivity of the two columns ensures a smooth transition even for quality control (QC) applications.

### Learn more about our Capto HiRes ion exchange chromatography columns. Click *here* for more information.



New-generation IEX columns

າo Q™ and

Highest resolution in lab-scale

urification

Transition

nade easy

Resin	Format	Description	Volume	Pack size	Item
Ni Sepharose excel.	Pre-packed columns	HisTrap excel 5 × 1 mL	1 mL/column	1/pk	17371205
Ni Sepharose excel.	Pre-packed columns	HisTrap excel 5 × 5 mL	5 mL/column	1/pk	17371206
StrepTactin Sepharose	Pre-packed columns	StrepTrap HP 5 × 1 mL	1 mL/column	1/pk	28907546
StrepTactin Sepharose	Pre-packed columns	StrepTrap HP 5 × 5 mL	5 mL/column	1/pk	28907548
MabSelect SuRe	Pre-packed columns	HiTrap MabSelect Sure 1 × 1 mL	1 mL/column	1/pk	29049104
MabSelect SuRe	Pre-packed columns	HiTrap MabSelect Sure, 1 × 5 mL	5 mL/column	1/pk	11003494
MabSelect PrismA	Pre-packed columns	HiTrap MabSelect PrismA 1 × 1 mL	1 mL/column	1/pk	17549851
Capto Q ImpRes	Pre-packed columns	HiTrap Capto Q ImpRes 5 × 1 mL	1 mL/column	1/pk	17547051
Capto Q ImpRes	Pre-packed columns	HiTrap Capto Q ImpRes 5 × 5 mL	5 mL/column	1/pk	17547055
Capto SP ImpRes	Pre-packed columns	HiTrap Capto SP ImpRes 5 × 1 mL	1 mL/column	1/pk	17546851
Capto SP ImpRes	Pre-packed columns	HiTrap Capto SP ImpRes 5 × 5 mL	5 mL/column	1/pk	17546855
Capto S ImpAct	Pre-packed columns	HiTrap Capto S ImpAct 5 × 1 mL	1 mL/column	1/pk	17371751
Capto S ImpAct	Pre-packed columns	HiTrap Capto S ImpAct 5 × 5 mL	5 mL/column	1/pk	17371755
Capto S ImpAct	Pre-packed columns	HiScreen Capto S ImpAct	4.7 mL/column	1/pk	17371747
HIC Resin	Pre-packed columns	HiTrap HIC Selection Kit, 7 × 1 mL	1 mL/column	1/pk	28411007
Capto HIC	Pre-packed columns	HiTrap Capto HIC Selection Kit 5 × 1 mL	1 mL/column	1/pk	29321087
Superdex 75 prep grade	Pre-packed columns	HiLoad 16/600 Superdex 75 pg	320 mL/column	1/pk	28989333
Superdex 200 prep grade	Pre-packed columns	HiLoad 16/600 Superdex 200 pg	120 mL/column	1/pk	28989335
Superose 6 prep grade	Pre-packed columns	HiLoad 16/600 Superose 6 pg	120 mL/column	1/pk	29323952
Superdex 30 Increase	Pre-packed columns	Superdex 30 Increase 10/300 GL	24 mL/column	1/pk	29219757
Superdex 75 Increase	Pre-packed columns	Superdex 75 Increase 10/300 GL	24 mL/column	1/pk	29148721
Superdex 200 Increase	Pre-packed columns	Superdex 200 Increase 10/300 GL	24 mL/column	1/pk	28990944
Capto HiRes Q	Pre-packed columns	Capto HiRes Q 5/50	1 mL/column	1/pk	29275878
Capto HiRes S	Pre-packed columns	Capto HiRes S 5/50	1 mL/column	1/pk	29275877
Sephadex G-25	Pre-packed columns	HiTrap Desalting, 5 × 5 mL	5 mL/column	1/pk	17140801
Sephadex G-25	Pre-packed columns	HiPrep 26/10 Desalting	53 mL/column	1/pk	17508701



# **DIBE™ technology for reliable HCP detection**

Host cell protein (HCP) is a primary impurity and a critical quality attribute (CQA) for biopharmaceuticals (biologics). HCP affects product quality, safety and efficacy. HCP ELISA is the gold standard of HCP detection and measurement, which requires polyclonal Antibodies (Ab) with broad reactivity against a wide range of potential HCPs.

Regulatory authorities require the characterization of the ELISA Abs used in the HCP ELISA assay. 2-D gel electrophoresis followed by Western blotting is the recommended approach to characterize HCP ELISA antibodies and their coverages.

2D differential in blot electrophoresis (2D DIBE) combined with Western blotting is a powerful technology for separation and visualization of complex protein mixtures such as HCPs.

High sensitivity—Fluorescent multiplexed methodology based on CyDye<sup>™</sup> pre-labeled Western blotting, and image acquisition with Amersham<sup>™</sup> Typhoon<sup>™</sup> laser scanner deliver high sensitivity for HCP detection.

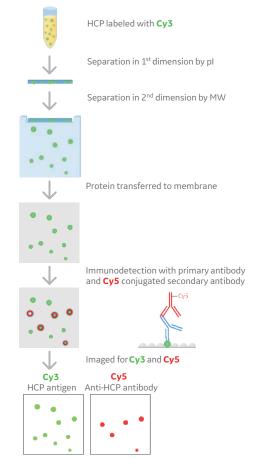
Minimal variation—Labeled proteins can be directly compared to the proteins detected by CyDye pre-labeled antibodies on the same membrane.

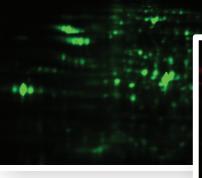
No mismatches—Multiplex fluorescence image acquisition with the Amersham Typhoon simultaneously captures both HCP antigen and anti-HCP antibody images from a single membrane. Fast evaluation—Melanie<sup>™</sup> Coverage software helps investigators evaluate the data in less time, with greater confidence.

#### 2D DIBE for HCP coverage assay

Total HCPs are labeled with CyDye DIGE Cy™3 minimal dye. After 2D electrophoresis, protein spots are transferred onto a PVDF membrane. The HCP antibody is applied to the membrane and visualized by Western blot with Cy5 fluorescence. Those two images are then overlaid. Cy3 labeled total HCP spot and Cy5 immunodetected spot overlay is confirmed by Melanie Coverage analysis software with 3D visualization. Finally, Melanie Coverage software provides a coverage percentage value for this assay.

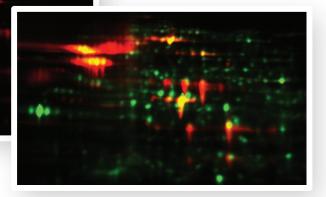
With the goal of helping you achieve the best results, we deliver 2D DIBE products that improve data quality when compared to traditional 2D experiments and Western blotting, and can be integrated into a complete HCP analysis solution.





Cy3 color image

Cy5 color image



Cy3 and Cy5 color overlay

### Amersham ECL<sup>™</sup> detection reagents

ECL based on horseradish peroxidase (HRP)-conjugated secondary antibodies has become the most commonly used detection method for Western blotting. It is a sensitive detection method, where the light emission is proportional to protein quantity. Minute quantities of proteins can be detected and quantitated.

Longer shelf life: up to 18 month shelf life on ECL Select<sup>™</sup> and Prime products.

 Stability: ECL Select and ECL Prime products are stable and stored at room temperature.

### Amersham Hyperfilm<sup>™</sup> ECL detection film

This is a sensitive film for the detection of chemiluminescent signals in Western blotting assays.

- Clear background for excellent contrast and band visibility.
- Publication-quality images.
- Learn more here: gelifesciences.com/wbfaq.

#### Amersham Western blotting membranes

We offer a broad selection of nitrocellulose (NC) and polyvinylidene difluoride (PVDF) Western blotting membranes, with pore size ranges to suit your application requirements.

- Optimized for chemiluminescent and fluorescent detection.
- Excellent protein binding capacity over a wide size range.
- New larger pack sizes reduce your price per blot by up to 30%.

### **CyDye labeling reagents**

CyDye Fluors are fluorescent dyes used in applications such as microarray analysis, FISH, 2-D DIGE, immunoprecipitation, and blotting.

Dyes are packaged in premixed amounts and foil-sealed to ensure consistent labelings.









For complete list of products to support your western blotting applications click here.



Chemistry	Format	Description	Volume/size	Pack size	Item
Chemiluminescent	Kit	ECL Western blotting detection reagent	For 2000 cm <sup>2</sup> membrane	1/pk	RPN2209
Chemiluminescent	Kit	ECL Select Western blotting detection reagent	For 1000 cm <sup>2</sup> membrane	1/pk	RPN2235
Chemiluminescent	Kit	ECL Prime Western blotting detection reagent	For 3000 cm <sup>2</sup> membrane	1/pk	RPN2236
Chemiluminescent	Kit	QuickStain kit	1 μg/mL to 20 mg/mL	1/pk	RPN4000
Chemiluminescent	Kit	Full range Rainbow molecular weight marker	250 µL	1/pk	RPN800E
Chemiluminescent	Sheets	Amersham Hyperfilm ECL	5 × 7 inches	50/pk	28906835
Chemiluminescent	Roll	Amersham Hybond™ PVDF membrane	0.2 µm, 260 mm × 4 m	1 roll	10600021
Chemiluminescent	Roll	Amershan Protran™ supported NC membrane	0.45 µm, 300 mm × 4 m	1 roll	10600016
Fluorescent labeling	Kit	Amersham CyDye Value Packs - Cy5 Mono - NHS Ester	10 mg	1/pk	PA15104
Fluorescent labeling	Kit	Amersham CyDye Value Packs - Cy7 Mono - NHS Ester	10 mg	1/pk	PA17104





GE, the GE Monogram, ÄKTA, Amersham, Anotop, Benchkote, Capto, Cell Boost, Cy, CyDye, DIBE, ECL, ECL Select, Ficoll, Ficoll-Paque, HiLoad, HiPrep, HiScreen, HisTrap, HiTrap, Hybond, HyClone, Hyperfilm, MabSelect, MabSelect SuRe, Mini-UniPrep, Mono Q, Mono S, Percoll, Protran, ReZist, Sepax, Sephacryl, Sera-Mag, SPARTAN, Typhoon, Uniflo, and Whatman GD/X are trademarks of General Electric Company. Agilent is a trademark of Agilent Technologies, Inc. TALON is a trademark of Clontech Laboratories Inc. Melanie is a trademark of SIB Swiss Institute of Bioinformatics. All other third-party trademarks are the property of their respective owners. © 2020 General Electric Company.

All offers in this issue are valid until 31 December 2020.

<sup>+</sup> The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F Hoffmann-La Roche Ltd. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as GE Healthcare and affiliates when used in conjunction with an authorized thermal cycler. Phi 29 DNA polymerase and its use for DNA synthesis is covered by US patent numbers 5,854,033, and 5,576,204. Ready to Go RT-PCR Beads: Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, and 6,127,155. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under

Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information. Every effort will be made to give reasonable notice of price changes but we reserve the right to change prices without further notice. All prices exclude value added or sales tax and may be subject to change without notice. These offers cannot be used in conjunction with any previously arranged purchase agreement.

KA6832060220BR

Austria: +43(0)800-20 88 40 Belgium: +32 (0)56 260 260 Denmark: +45 70 27 99 20 Germany: +49 (0)2304 9325 Ireland: +353 (0)1 885 5854 Italy: +39 02 950 59 478 Finland: +358 (0)9 8027 6280 France: +33 (0)3 88 67 14 14 Netherlands: +31 (0)20 487 70 00 Norway: +47 22 95 59 59 Portugal: +351 21 425 33 50 Spain: +34 902 239 303 Sweden: +46 31 352 32 00 Switzerland: +41 (0)56 618 41 11 UK: +44 (0)1509 555 500

