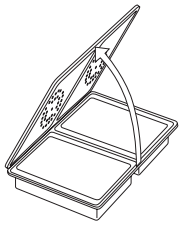
SNAP i.d.™ OVERVIEW OF PROCEDURE

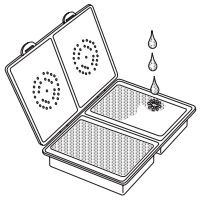
Before using the SNAP i.d. Protein Detection System, please read the User Guide completely.

1. Open the blot holder lid, taking care not to damage the inner white surface.



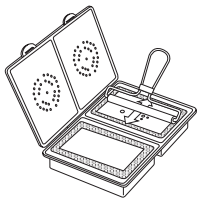
2. Thoroughly wet the white surface of the blot holder with Milli-Q® water.

   NOTE: If using only one well of a double or triple well blot holder, the unused well(s) must also be wet.

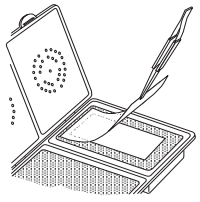


3. Place the pre-wet blot in the center of the blot holder with the protein side down. The blot membrane should not exceed size specified in the User Guide.

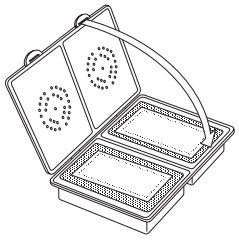
4. Roll blot membrane gently to remove air bubbles.



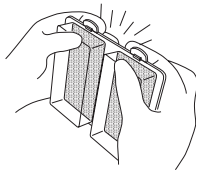
5. Place the spacer (wetting not necessary) on top of the blot membrane and roll again to ensure contact of spacer with blot membrane.



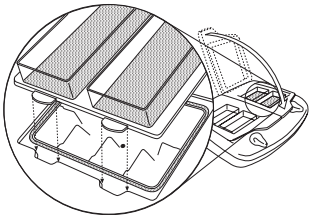
6. Close the blot holder lid.



7. Squeeze firmly at base of tab area to secure lid.

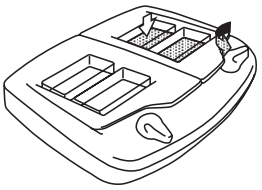


8. Open lid of system and place blot holder in chamber, aligning blot holder tabs with notches of chamber. Close and latch lid.



9. Add volume of blocking solution as indicated under **OPTIMIZATION GUIDELINES** on reverse side. Using knobs on the system, apply vacuum until well(s) are completely empty.

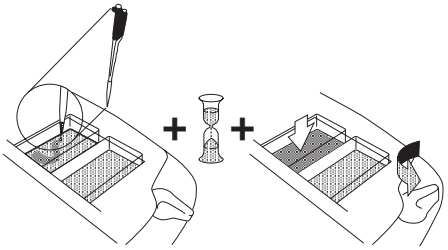
   TURN VACUUM OFF.



10. Add volume of primary antibody as indicated under **OPTIMIZATION GUIDELINES** on reverse side. Antibody solution must evenly cover ***entire*** blot holder surface.

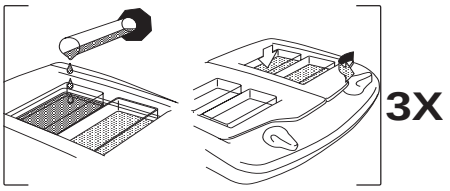
    Incubate for 10 minutes at room temperature. Solution will be absorbed into the blot holder and surface may appear dry. Apply vacuum.

    IMPORTANT: Do not apply vacuum until after the 10-minute incubation.



11. With vacuum running continuously, wash 3 times with wash buffer. See **OPTIMIZATION GUIDELINES** on reverse side for volumes.

    TURN VACUUM OFF.



12. Apply appropriate volume of secondary antibody (see **OPTIMIZATION GUIDELINES** on reverse side) evenly across the blot holder surface. Incubate for 10 minutes at room temperature. Again, solution will be absorbed into the blot holder and surface may appear dry. Apply vacuum.

    IMPORTANT: Do not apply vacuum until after the 10-minute incubation.

13. With vacuum on, wash 3 times with buffer. See **OPTIMIZATION GUIDELINES** on reverse side for volumes.

    TURN VACUUM OFF.

14. Remove blot and incubate with the appropriate detection reagent such as Immobilon® HRP, or, if using Millipore fluorescently labeled antibodies, visualize.

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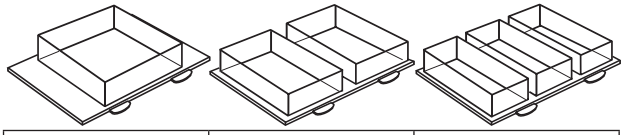
MILLIPORE™

SNAP i.d.™ OPTIMIZATION GUIDELINES

Blocking, Antibody and Wash Recommended Volumes



	Single well	Double well	Triple well
Blocking solution volume	30 mL/well	15 mL/well	10 mL/well
Antibody volume	3 mL/well	1.5 mL/well	1 mL/well
Wash buffer* volume	30 mL/well	15 mL/well	10 mL/well

** Tris or phosphate buffered saline solutions, supplemented with 0.1% Tween® 20 surfactant.*

It is not necessary to use all the wells of double and triple well blot holders, but unused wells must be wet out with Milli-Q water.

Blot Blocking Concentration

- **The use of non-fat/low fat dry milk at concentrations higher than 0.5% is not recommended, as this will result in clogging of the blot holder and prevent reagent flow.**
- Blocking agents should be prepared in tris or phosphate buffered saline solutions containing 0.1% Tween 20 surfactant, to reduce surface tension and ensure even distribution of blocking agent across the blot holder surface.
- The SNAP i.d. system is compatible with the most commonly used blocking agents. Refer to User Guide for complete list with recommended concentrations.
- In order to insure optimal flow through the blot holder, it is essential that blocking solutions be completely solubilized and free of all particulate matter. In some cases, it may be necessary to reduce the concentration of the blocking agent to achieve the required flow.

Antibody Volume and Concentration

- **Most users will be able to use the same amount of antibody, but in 1/3 to 1/5 the volume at 3–5 fold higher concentration.**

	Standard Immunodetection	SNAP i.d. Immunodetection
Mass of antibody required	**1 µg**	**1 µg**
Stock solution	1 mg/mL	1 mg/mL
Diluted stock	1:10,000 (0.1 µg/mL)	1:3,333 (0.33 µg/mL)
Volume required for assay	10 mL	3 mL
Antibody used	0.1 µg/mL × 10 mL = 1 µg	0.33 µg/mL × 3 mL = 1 µg

This guideline is intended as a starting point to develop the final antibody concentration necessary for desired performance. Because each antibody is different, it may be necessary to adjust the blot exposure time, antigen load or both.