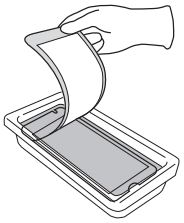
SNAP i.d.® 2.0 Blot Holder and Frame

Instructions for Use with the SNAP i.d.® 2.0 Protein Detection System

For research use only. Not for use in diagnostic procedures.

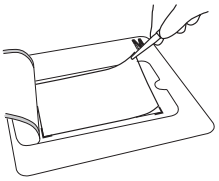
Before using the SNAP i.d.® 2.0 Protein Detection System, please read the full length user guide.

1. Hold the blot holder by the support layer (blue edges) and wet the membrane layer (white) with distilled water in the wetting tray provided. Do not wet the support layer. Place the wetted blot holder on the rolling pad.

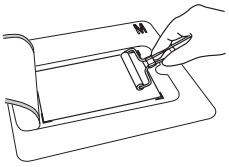


2. If required, pre-wet the blot in methanol and water, then place it in the center of the blot holder with the **protein side down**.

   NOTE: Blot should not exceed size specified in the user guide.

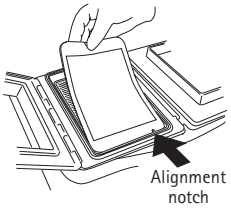


3. Roll the blot gently to remove air bubbles, then close the blot holder and roll one more time.



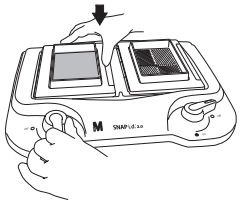
4. Open the blot holder frame, flip the blot holder so that it is **protein side up**, then place it inside the frame. A notch in the blot holder ensures correct placement in the frame.

   NOTE: If running only one MultiBlot in the frame, place the well blank card in the second well.

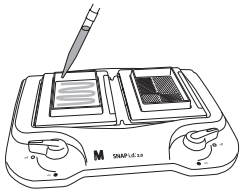


5. Close and lock the frame. Add 30 mL of blocking solution (15 mL for MultiBlot). Press the frame down and turn the system knob to apply vacuum.

   When frame is completely empty, **TURN VACUUM OFF**.

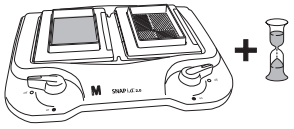


6. Apply appropriate volume of primary antibody across the surface of the blot holder (2.5 mL for MultiBlot, 5 mL for Mini blot, or 10 mL for Midi blot).

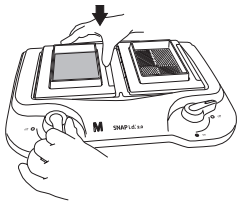


7. Incubate for 10 minutes at room temperature. Solution will be absorbed into the blot holder and surface may appear dry.

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

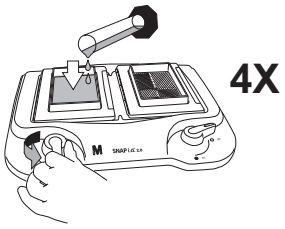


8. Press the frame down and apply vacuum. Wait 5–8 seconds until the frame is completely empty.



9. With vacuum running continuously, add 30 mL of wash buffer (15 mL for MultiBlot). Repeat the washing step 3 more times (total of 4 washes).

   When frame is completely empty, **TURN VACUUM OFF**.



10. Apply appropriate volume of secondary antibody across the surface of the blot holder (2.5 mL for MultiBlot, 5 mL for Mini blot, or 10 mL for Midi blot). Incubate for 10 minutes at room temperature with vacuum off. Again, solution will be absorbed into the blot holder and surface may appear dry.

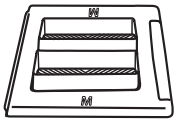
    IMPORTANT: Do not apply vacuum until after the 10-minute incubation.

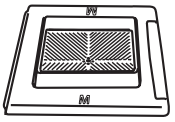
11. Press frame down and apply vacuum. Wait 5–8 seconds until frame is completely empty. With vacuum running continuously, add 30 mL of wash buffer (15 mL for MultiBlot). Repeat the washing step 3 more times (total of 4 washes).

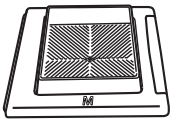
12. Turn vacuum off and remove blot holder from frame. Remove blot from blot holder and incubate with the appropriate detection reagent. If the MultiBlot well blank was used, remove and clean.

SNAP i.d.® 2.0 Optimization Guidelines

Blocking, Antibody, and Wash Recommended Volumes







	SNAP i.d.® 2.0 MultiBlot Frame	SNAP i.d.® 2.0 Mini Frame	SNAP i.d.® 2.0 Midi Frame
Blocking solution volume	15 mL	30 mL	30 mL
Antibody volume	2.5 mL	5 mL	10 mL
Wash buffer* volume	4 × 15 mL each	4 × 30 mL each	4 × 30 mL each

** Tris- or phosphate-buffered saline solution, supplemented with 0.1% Tween® 20 surfactant*

Blot Blocking

- The SNAP i.d.® 2.0 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.
- The use of non-fat/low fat dry milk at concentrations higher than 0.5% is **not recommended.**
- Blocking agents should be prepared in tris- or phosphate-buffered saline solution containing 0.1% Tween® 20 surfactant, to reduce surface tension and ensure even distribution of blocking agent across the blot holder surface.
- To ensure even distribution of the antibody in the incubation step, dilute the antibody in blocking solution that contains Tween® 20 surfactant.

Antibody Volume and Concentration

Most users will be able to use the same amount of antibody, but in less volume at a higher concentration. See example below.

	Standard Immunodetection	SNAP i.d.® 2.0 Immunodetection		
		MultiBlot	Mini Blot	Midi Blot
Antibody stock concentration	1 mg/mL	1 mg/mL	1 mg/mL	1 mg/mL
Mass of antibody required	1 µg	0.25 µg	0.5 µg	1 µg
Volume of antibody used	30 mL	2.5 mL	5 mL	10 mL
Final antibody dilution	1:30,000	1:10,000	1:10,000	1:10,000
Antibody stock used	1 µL	0.25 µL	0.5 µL	1 µL

This guideline is intended as a starting point to develop the final antibody concentration for desired performance. Because each antibody is unique, it may be necessary to adjust the antibody and antigen concentrations, the type and/or sensitivity of the detection reagent used, or the blot exposure time.

Product Ordering Information

Description	Cat. No.	Qty/Pk
SNAP i.d.® 2.0 MultiBlot Frame	SNAP2FRMB01	MultiBlot frame with lid (1) MultiBlot holders (2)
SNAP i.d.® 2.0 Mini Frame (single pack)	SNAP2FRMN01	Mini frame with lid (1) Mini blot holders (2)
SNAP i.d.® 2.0 Mini Frame (double pack)	SNAP2FRMN02	Mini frame with lid (2) Mini blot holders (4)
SNAP i.d.® 2.0 Midi Frame (single pack)	SNAP2FRMD01	Midi frame with lid (1) Midi blot holders (2)
SNAP i.d.® 2.0 Midi Frame (double pack)	SNAP2FRMD02	Midi frame with lid (2) Midi blot holders (4)
SNAP i.d.® 2.0 MultiBlot Holders (includes 2 well blanks)	SNAP2BHMB050	50
SNAP i.d.® 2.0 Mini Blot Holders	SNAP2BHMN0100	100
SNAP i.d.® 2.0 Midi Blot Holders	SNAP2BHMD0100	100

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Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (within the "Terms and Conditions of Sale" applicable to your purchase transaction).

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