User Guide

SNAP i.d.® 2.0 Protein Detection System for Immunohistochemistry

Notice

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00001195, Rev. A, 06/14

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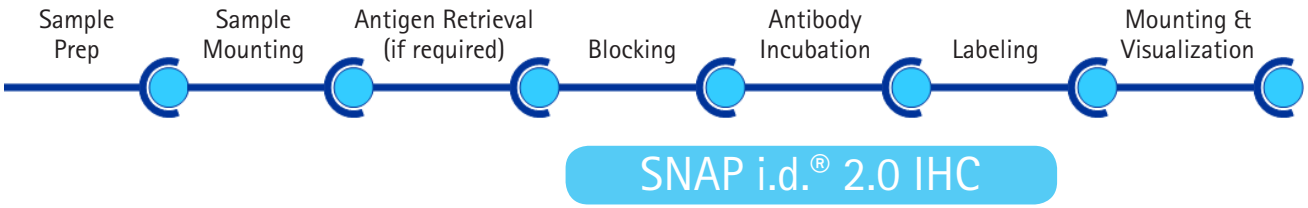
Introduction

SNAP i.d.® 2.0 Protein Detection for Immunohistochemistry (IHC) introduces a new capability to the SNAP i.d.® 2.0 system. An IHC frame and specially designed slide holders allow the user to block, probe, and stain up to 12 tissue slides at a time. The system accommodates two frames, thereby allowing the user to process up to 24 slides simultaneously. It can handle both frozen and paraffin-embedded samples, but cannot be used for mounting, deparaffinization, rehydration, or antigen retrieval steps. Reduced handling time and multiple sample capability make this vacuum-driven system ideal for antibody and protocol optimization.



Features of the SNAP i.d.® 2.0 Protein Detection System for IHC

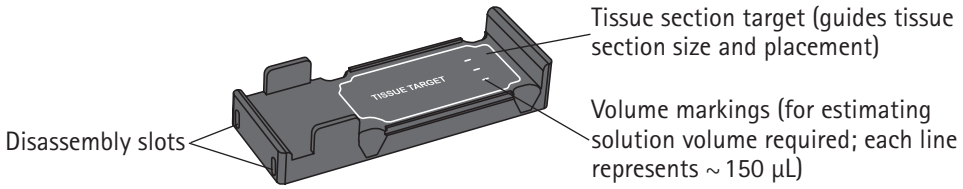
- Vacuum base with two individually controlled sides allows independent processing of one or two IHC frames.
- Each IHC frame can process 1 to 12 glass slides through independent vacuum ports. Empty ports can be plugged when not in use.
- IHC frames have covers and can be removed from the base for extended incubation (one hour to overnight) at 4 °C. Covered frames can be stacked for off-line processing.
- Task tracker feature on IHC frame cover helps keep track of IHC steps.
- Disposable slide holder is designed for standard 25 x 75 x 1 mm glass slides. An antibody injection/recovery port facilitates addition and removal of small volumes of antibodies or reagents.
- Assembly fixture aids in assembly and disassembly of slide and slide holder.
- Slide holder wash reservoir can hold up to 6 mL of wash solution or blocking buffers.
- Greater than 75% antibody recovery via the injection/recovery port.
- Easily adaptable to a variety of immunostaining protocols, the SNAP i.d.® 2.0 IHC System works with all blocking buffers, antibodies, and visualization methodologies (e.g., fluorescence or colorimetric).

The SNAP i.d.® 2.0 Protein Detection System is intended for research use only.

Components of the SNAP i.d.® 2.0 IHC System

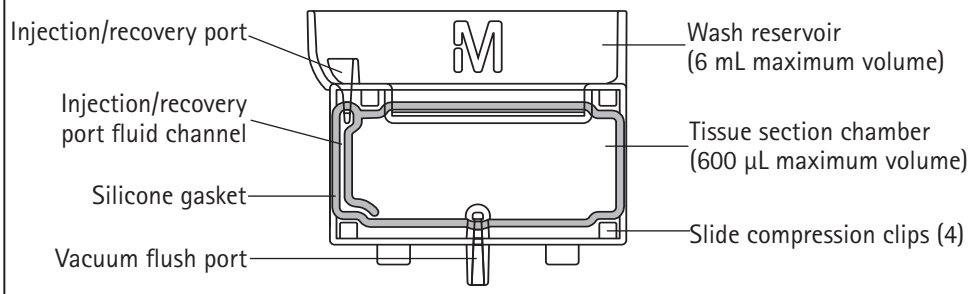
Assembly Fixture

Accepts 25 × 75 × 1 mm slide (slides not provided)



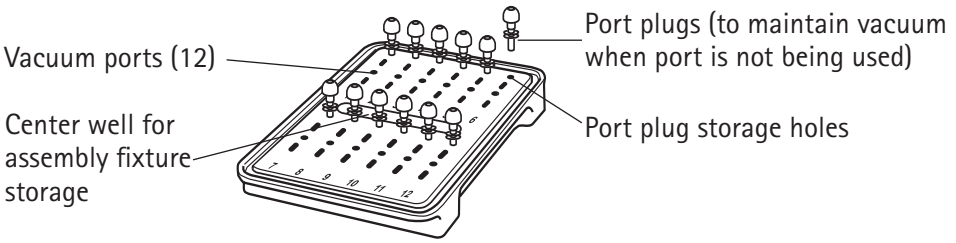
Slide Holder

Injection/recovery port accepts 1 mL pipette tip

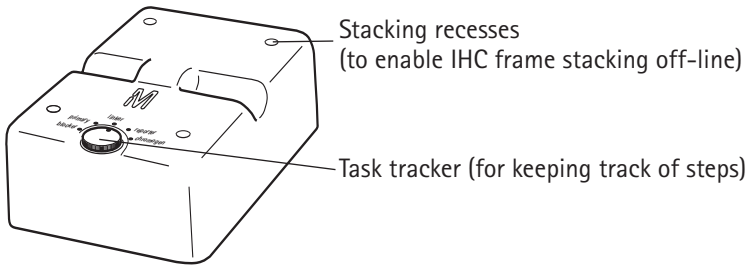


IHC Frame

Holds up to 12 slide holders



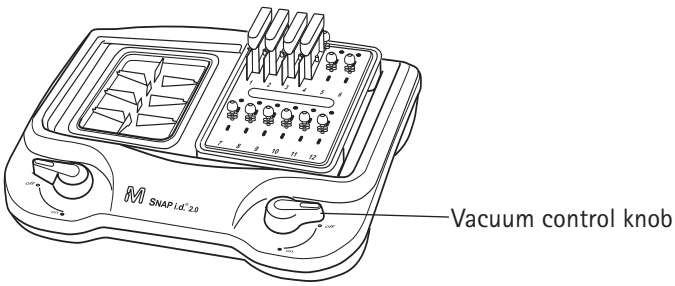
Incubation Cover



SNAP i.d.® 2.0 IHC System

Accommodates one or two IHC frames

Includes tubing assembly kit



Symbols Used in this User Guide

The following symbols are used throughout this user guide and/or on product labels, and the user shall abide by indicated requirements:

Symbol	Definition	Symbol	Definition
	Warning alerts you to actions that may cause personal injury or pose a physical threat.		Lot number
	Read the documentation		Manufacturer
	Catalogue number		Do not re-use
	Serial number		

Materials Required but Not Supplied

- Vacuum source: Pump or other uniform vacuum source that can deliver a sustained minimum pressure of 410 millibar (12 in. Hg) and 34 L/min. Refer to Ordering Information for pump catalogue numbers.

  NOTE: EMD Millipore Corporation's WP61 series Chemical Duty Pumps can be used, but may require longer processing times.
- One liter or larger vacuum flask with stopper (for waste collection). A Millex®-FA_{50} filter (or equivalent) is recommended between the vacuum flask and the vacuum source to protect the vacuum source from contamination.
- Vacuum tubing to connect vacuum flask to vacuum source
- Glass slide (25 x 75 x 1 mm) with tissue sample
- Pipettor with 1 mL pipette tips (do **not** use pipette tips with aerosol filters)
- Blocking reagents
- Antibodies
- Detection reagents
- Wash buffers

General Guidelines

System

- The SNAP i.d.® 2.0 IHC system can be adapted to any immunostaining protocol. It works with both fresh/frozen and paraffin-embedded samples, however, it is not designed to perform deparaffinization, rehydration, antigen retrieval, or dehydration steps.
- Operating temperature: 4–37 °C.
- Antibody incubation steps can be performed on the SNAP i.d.® 2.0 base or off-line.
- To ensure full vacuum, plug all unused vacuum ports with the blue plugs provided.

Tissue Preparation

- Good adherence of the tissue sample to the glass slide is critical to system performance.
- Tissue thickness should not exceed 20 µm.
- Before placing tissue on slide, make sure that it will not extend beyond the target area of the assembly fixture.

Assembly of Glass Slide and Slide Holder

- **IMPORTANT!** Do not touch the gasket on the slide holder during assembly.
- Slide holder accepts only 25 x 75 x 1 mm slides
- Ensure that the tissue sample does not extend beyond the tissue target area outlined on the assembly fixture. Tissue extending into the gasket area will compromise the seal between the slide holder and slide, resulting in leakage. In addition, tissue extending outside the gasket area will not be stained, and may be lost during slide processing.
- Once the glass slide is assembled in the slide holder, ensure that all four clips are fully engaged prior to adding liquids (refer to Slide Holder Setup section).
- After assembly do not try to reposition the slide in the slide holder, as this may damage the gasket and cause leakage.

Reagents

- The system requires 150–600 µL of antibody or chromogen. To ensure complete tissue staining, these reagents must completely cover the tissue area.
- Because each antibody is unique and the sensitivity of detection reagents varies, it may be necessary to adjust the antibody concentration and/or incubation time. Refer to manufacturer antibody instructions for specific recommendations.
- Some chemicals used in the immunostaining process are carcinogenic or toxic. Refer to applicable international, federal, state, and local regulations for appropriate disposal.

Reagents, continued

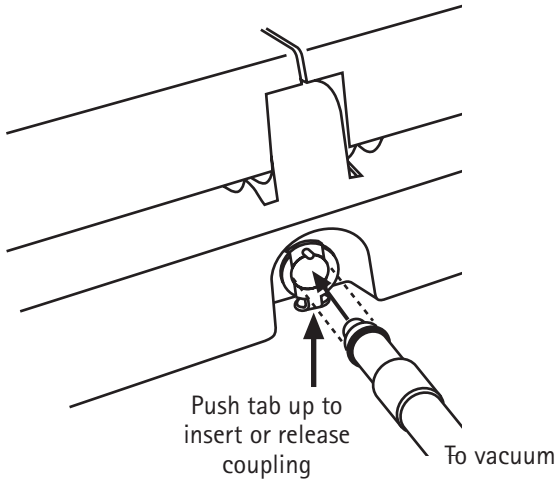
- For uniform staining of the tissue sample, it is critical to avoid bubbles during the reagent loading steps. Bubbles can form when reagents are dispensed too quickly through the injection/recovery port, or if air is introduced by the pipette after adding reagent.
- Three to four washes of up to 6 mL each are recommended to ensure complete washing of the tissue after each incubation step. A squirt bottle can be used to facilitate the addition of wash buffer to the wash reservoir.
- Tissue staining with DAB and hematoxylin can be performed in the SNAP i.d.® 2.0 IHC system, however, these chemicals can permanently stain the base.
- Blocking, Antibody, and Wash Recommended Volumes

Solution	Volume	Comments
Blocking solution	600–6,000 µL	Depending on the protocol, blocking volumes can vary. If your protocol calls for small volumes, insert the solution through the injection/recovery port. For protocols calling for larger volumes, add the solution through the wash reservoir.
Antibody	150–600 µL	Make sure the antibody volume is sufficient to cover the tissue sample completely.
Wash buffer	3 to 4 washes, up to 6 mL each wash	Tris- or phosphate-buffered saline solutions, supplemented with 0.05% Tween® 20 surfactant (TBST or PBST)

How to Set Up the SNAP i.d.® 2.0 System

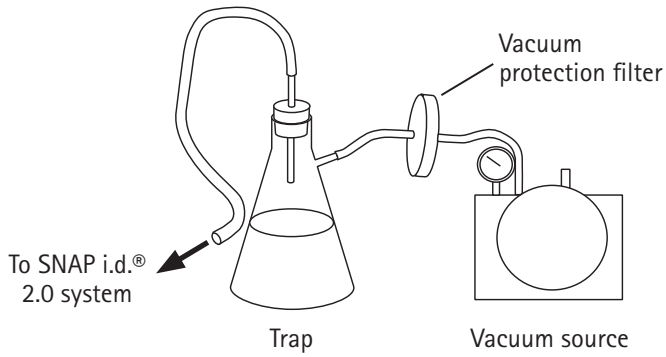
1. Place the SNAP i.d.® 2.0 base on a level bench top.

2. Attach the vacuum tubing to the back of the system by pushing the coupling insert on the end of the tubing into the quick disconnect fitting at the back of the system base until it clicks.



NOTE: To disconnect the tubing, push the tab below the tubing connector up with the index finger and pull tubing out.

3. Connect the other end of the tubing to a vacuum source. Use a one-liter vacuum flask as a trap and a Millex®-FA_{50} filter (cat. no. SLFA05010) to protect the vacuum source from contamination, as shown below.



NOTE: Any vacuum source that can deliver 410 millibar (12 in. Hg) and 34 L/min is sufficient. If the vacuum source operates at higher than 410 millibar, the SNAP i.d.® 2.0 system will automatically regulate the vacuum pressure.

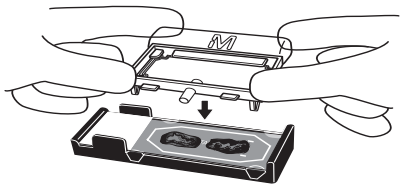
If the vacuum source is insufficient, the flow rate through the system may be inconsistent, resulting in longer processing times.

How to Use the SNAP i.d.® 2.0 IHC System

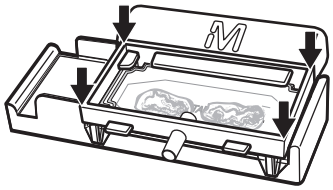
Slide Holder Setup

1. Place assembly fixture on a hard, flat surface. Position slide in assembly fixture, with the tissue section facing up. Verify that the tissue section does not extend beyond the target area.

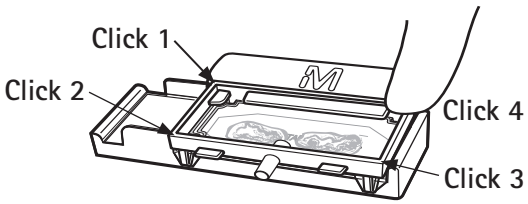
2. Hold the slide holder by the sides, clip side down, and align it with the assembly fixture containing the slide. **Do not touch the gasket**.



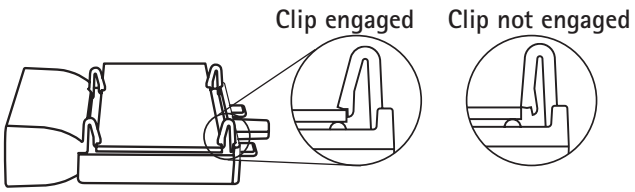
3. Press the slide holder down firmly over the slide until the four compression clips slip over the slide edges with an audible click.



4. To ensure that all clips are fully engaged, firmly press down on the top edge of each slide holder corner, one at a time, and listen for a click on each press.



5. Remove the slide holder assembly from the fixture and closely inspect each clip to confirm that it is in the proper position over the slide. If any clip is not engaging the slide as shown below, place the assembly back into the fixture and repeat step 4 on the unengaged clip(s).

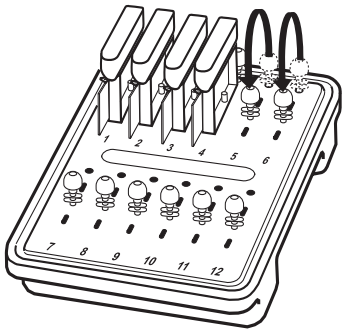


NOTE: Do not try to reposition the slide in the slide holder after assembly, as this may damage the gasket and cause leakage.

IHC Frame Setup

The IHC frame can be set up either on or off the SNAP i.d.® 2.0 system.

1. Place assembled slide holders into any vacuum ports on the frame, with slide labels oriented towards the port numbers.



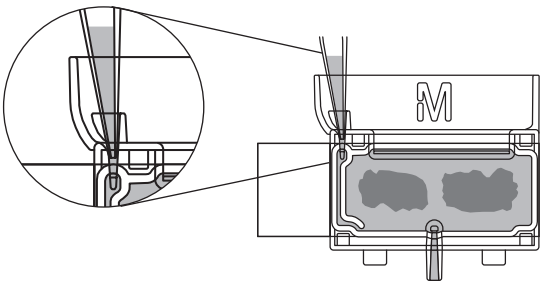
2. Press blue port plugs **lightly** into all unused ports (excessive force is not required). Unused plugs can be left in the port plug storage holes.

3. If the frame has been assembled off the SNAP i.d.® 2.0 system, place it in either position on the system before continuing.

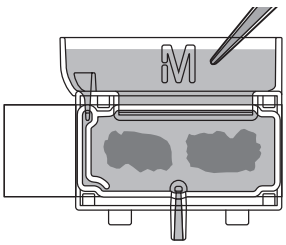
Staining Protocol

Tips for adding solutions to slide holder

- Small volumes (up to 600 µL) should be dispensed with a 1 mL pipette through the injection/recovery port. **Gently** push the pipette tip into the port to create a seal. Dispense slowly to avoid bubble formation in the tissue section chamber.



- Larger volumes (up to 6 mL) can be loaded into the wash reservoir.



- Make sure that the solution completely covers the tissue sections.

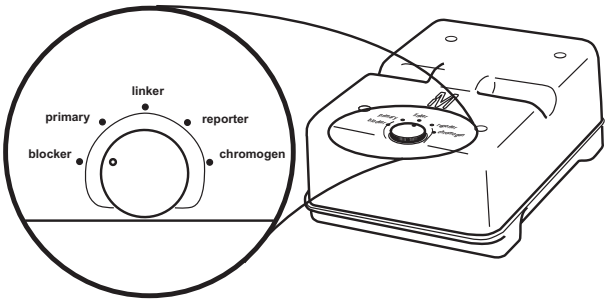
Washing (optional)

1. If washing is required before blocking, fill the wash reservoir of the slide holder with up to 6 mL of desired wash solution.
2. To flush the wash solution out of the tissue chamber, turn on the SNAP i.d.® 2.0 system vacuum.

   NOTE: Residual fluid at the bottom of the tissue section chamber will not impact subsequent steps.

Blocking

1. Load blocking reagent through the injection/recovery port or the wash reservoir.
2. If incubation is required, place the cover on the IHC frame and set the task tracker to **"blocker"**.



3. To flush the blocking solution from the slide holder, remove the cover and activate the vacuum.
4. If wash steps are needed, fill the wash reservoir with wash solution, then flush by applying vacuum. If necessary, repeat wash step two more times.

Primary Antibody

1. Inject up to 600 µL of primary antibody through the injection/recovery port, dispensing slowly to avoid bubbles, and ensuring complete coverage of the tissue section.
2. Cover the IHC frame and set the task tracker to **"primary"**. When incubation is complete, remove the cover.
3. Recover primary antibody with a pipette via the injection/recovery port or flush to waste by applying vacuum.
4. Fill the wash reservoir with wash solution, then flush by applying vacuum. Repeat wash step two more times if required.

Linker (secondary antibody)

1. Inject up to 600 µL of linker through the injection/recovery port, dispensing slowly to avoid bubbles, and ensuring complete coverage of the tissue section.
2. Cover the IHC frame and set the task tracker to **"linker"**. When incubation is complete, remove the cover.

Linker (secondary antibody), continued

3. Recover linker with a pipette via the injection/recovery port or flush to waste by applying vacuum.
4. Fill the wash reservoir with wash solution, then flush by applying vacuum. Repeat wash step two more times if required.

Reporter (tertiary, if required)

1. Inject up to 600 µL of reporter through the injection/recovery port, dispensing slowly to avoid bubbles, and ensuring complete coverage of the tissue section.
2. Cover the IHC frame and set the task tracker to "**reporter**". When incubation is complete, remove the cover.
3. Recover reporter with a pipette via the injection/recovery port or flush to waste by applying vacuum.
4. Fill the wash reservoir with wash solution, then flush by applying vacuum. Repeat wash step two more times if required.

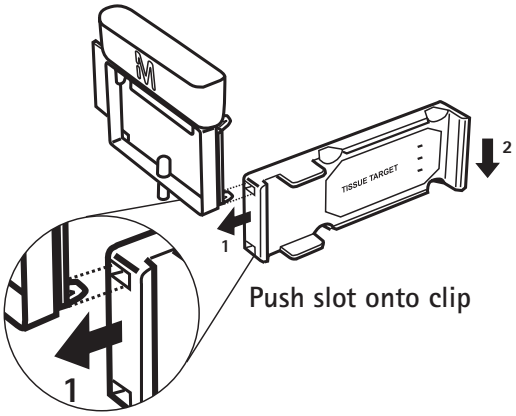
Chromogen

NOTE: Some stains such as hematoxylin and eosin may permanently stain the SNAP i.d.® 2.0 system.

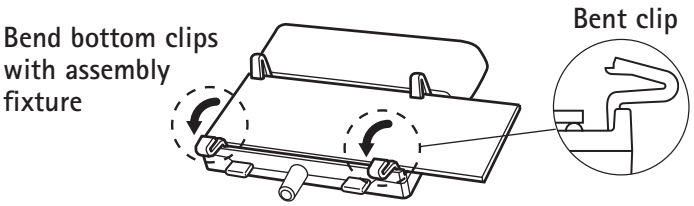
1. Inject up to 600 µL of desired chromogen through the injection/recovery port, dispensing slowly to avoid bubbles, and ensuring complete coverage of the tissue section.
2. Cover the IHC frame and set the task tracker to "**chromogen**". When incubation is complete, remove the cover.
3. Recover chromogen with a pipette via the injection/recovery port or flush to waste by applying vacuum.
4. Fill the wash reservoir with wash solution, then flush by applying vacuum. Repeat wash two more times if required.

Slide Removal

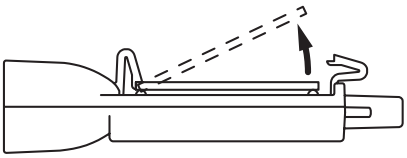
1. After staining/counter-staining is complete, a final wash is recommended before removing the slide from the slide holder.
2. To remove the slide, push the assembly fixture disassembly slot over one of the bottom clips (1).



3. Bend the clip 90 degrees away from the slide (2). Repeat with the other bottom clip.



4. Carefully lift the slide up and out of the holder.



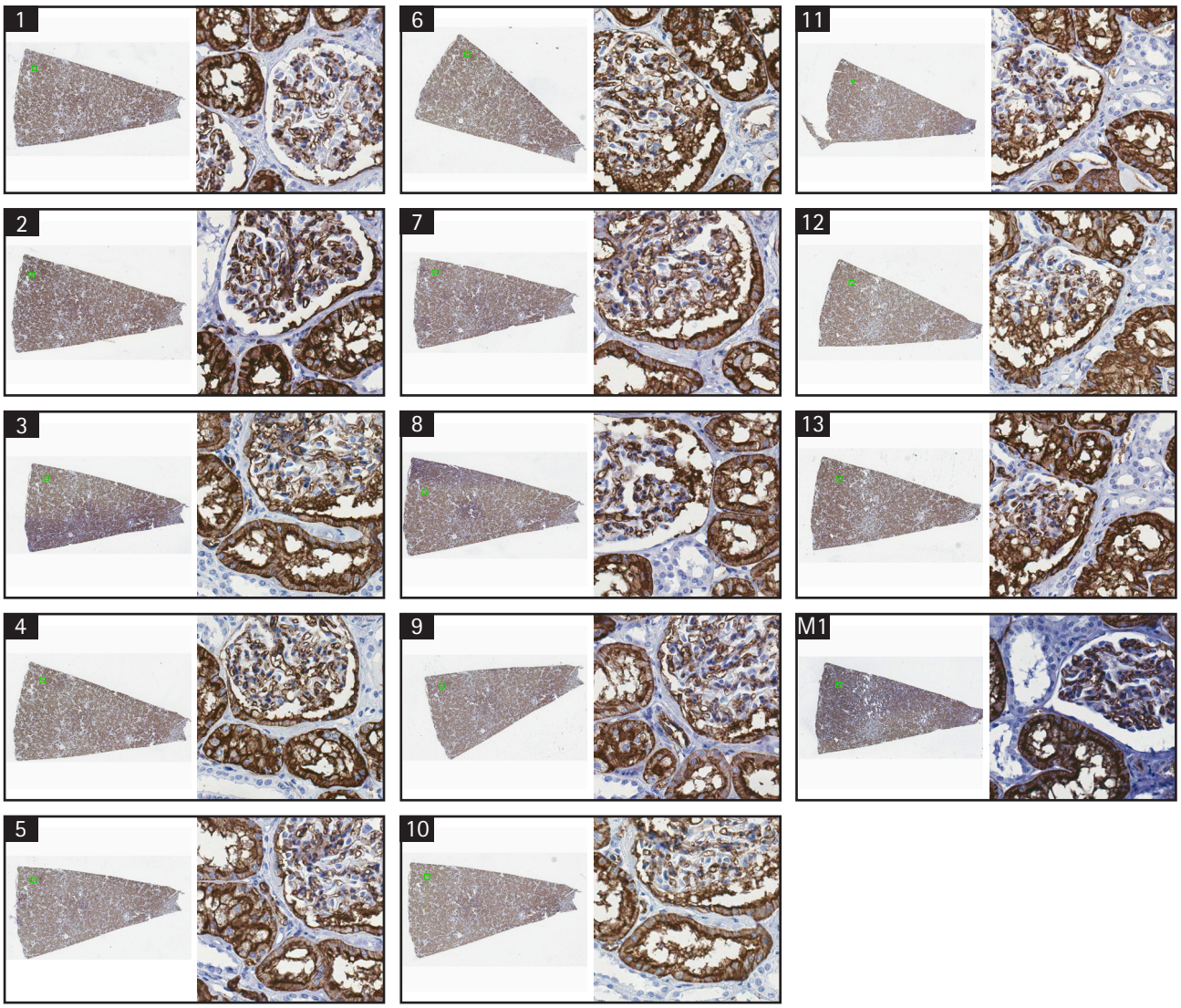
5. Discard the used slide holder. If required, proceed with dehydration and cover slip mounting protocols.

   NOTE: Do not use the SNAP i.d.® 2.0 system for dehydration or mounting steps.

Proof of Performance

Figure 1. SNAP i.d.® IHC System Demonstration of Staining Consistency

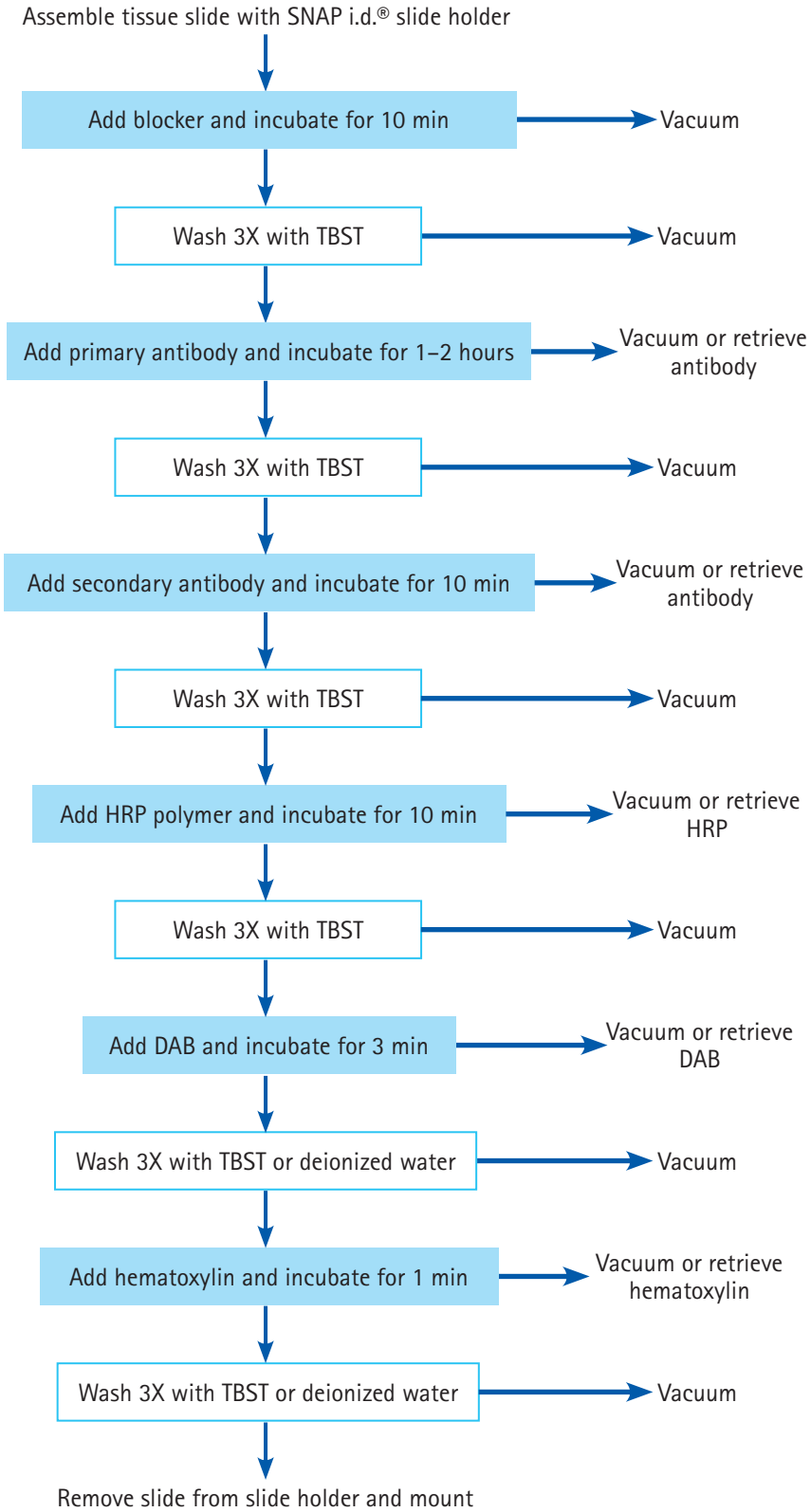
Detection of Aquaporin 1 in human kidney tissue (formalin-fixed and paraffin-embedded (FFPE)): SNAP i.d.® 2.0 IHC System (slides 1–13) vs. manual IHC protocol (slide M1)



Fourteen tissue sections (5 µm) were assembled on FisherBiotech® ProbeOn Plus™ slides, then underwent rehydration, deparaffinization, and HIER (heat-induced epitope retrieval) antigen retrieval prior to staining. Slides 1–13 were processed using the SNAP i.d.® 2.0 IHC immunostaining protocol outlined in Figure 2. Slides 1–12 were processed in parallel on one frame of the SNAP i.d.® IHC system and slide 13 was processed alone on another IHC frame. Slide M1 was processed using the manual protocol. Anti-Aquaporin 1 (cat. no. AB2219) was diluted 1:2,000.

Proof of Performance, continued

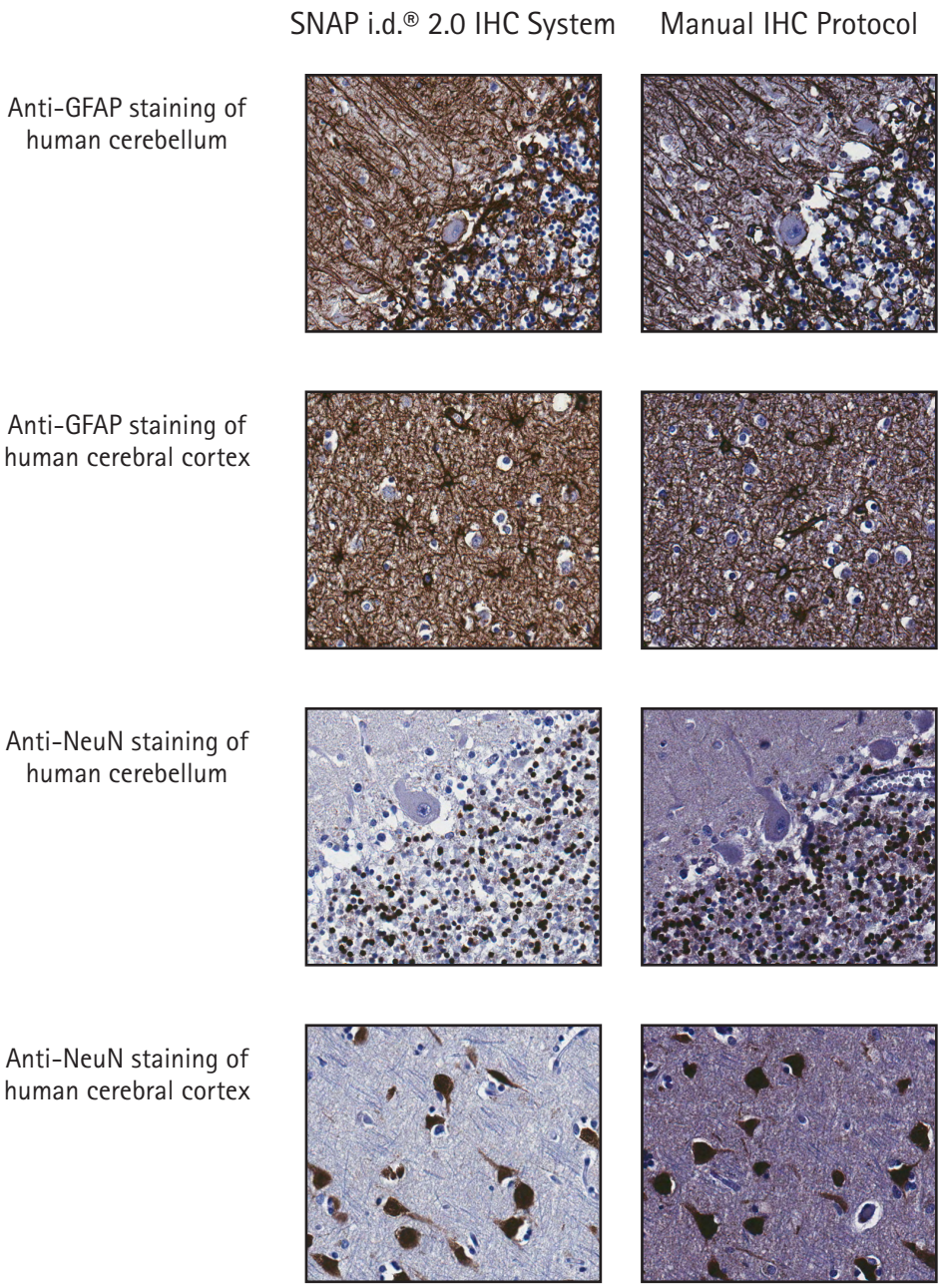
Figure 2. Typical SNAP i.d.® 2.0 IHC Immunostaining Protocol



Proof of Performance, continued

Figure 3. Comparison of Antibodies

Detection of glial fibrillary acidic protein (GFAP) and neuronal nuclei protein (NeuN) in human cerebellum and cerebral cortex tissues (FFPE): SNAP i.d.® 2.0 IHC System vs. manual IHC protocol



Tissue sections (5 µm) were assembled on FisherBiotech® ProbeOn Plus™ slides, then underwent rehydration, deparaffinization, and HIER antigen retrieval prior to staining. Slides were processed in the SNAP i.d.® 2.0 IHC System according to the protocol in Figure 2, or with the manual IHC protocol. Both Anti-GFAP (cat. no. MAB3402) and Anti-NeuN (cat. no. MAB377) were diluted 1:1,000.

Maintaining the SNAP i.d.® 2.0 System

Cleaning Protocol

The SNAP i.d.® 2.0 system **must** be cleaned after each use. Residual salts can crystallize, blocking vacuum ports and interfering with valve function. To remove salts or contaminants from the IHC frame, base, and tubing, turn the vacuum on and flush distilled water through the system.

NOTE: Do not autoclave the SNAP i.d.® 2.0 base or IHC frames.

Component Re-use

Slide holders are **single-use only**.

Refer to applicable international, federal, state, and local regulations for appropriate disposal.

Troubleshooting

Symptom	Cause	Corrective Action
Vacuum control knobs stick	Inadequate cleaning	Flush system with distilled water.
Solution does not empty from the slide holder	Inadequate vacuum	Make sure tubing connection between system and vacuum source is secure. If using two IHC frames, run one side at a time.
	Inadequate cleaning	Make sure all vacuum ports are clean and free of debris or salt.
		Empty the vacuum flask and change the in-line Millex®-FA_{50} filter.
	Slide holder not seated correctly in the vacuum port	Make sure slide holder is fully seated in the vacuum port.
	Unused vacuum ports not plugged	Make sure that every vacuum port in the frame (12 total) has either a slide holder or a port plug in it.
Slide holder leaks around gasket	Slide holder not completely sealed to the slide	Make sure that slide holder is completely sealed to slide, with all four compression clips engaged. Refer to Slide Holder Setup section. When placing slide on assembly fixture, position slide edge against the right side of the assembly fixture (away from the disassembly slots).
	Slide moved after assembly in slide holder, damaging the gasket and allowing solution to leak out	Do not try to reposition slide after assembly in slide holder.
Solution leaks out of vacuum flush port	Leaking valve(s) in IHC frame, due to salt build-up	Place the IHC frame on the SNAP i.d.® base and flush water through the frame with the vacuum on. Soak the IHC frame in water for 30 minutes to remove salt build-up on valves. If problem persists, contact Technical Service.

Troubleshooting, continued

Symptom	Cause	Corrective Action
Solution leaks out of vacuum flush port, continued	Pipette tip with aerosol filter was used to add solution to injection/recovery port, exerting additional pressure on the valve	Do not use pipette tips with aerosol filters.
Poor staining	Primary and/or secondary antibody concentration and/or volume too low	Increase the antibody concentration and/or increase volume to 600 µL.
	Antibody leaked outside the tissue section chamber	Make sure that slide is correctly assembled in the slide holder, and that the four compression clips are completely engaged. Refer to Slide Holder Setup section.
	Incubation time not long enough	Increase antibody incubation time.
High background	Inadequate blocking	Change to different blocking solution. Increase the blocking time.
	Blocking solution may have degraded	Always prepare fresh reagents.
	Antibody concentration too high	Decrease concentration of antibody.
	Inadequate washing	Run at least 4 washes of up to 6 mL each. Add wash solution to the wash reservoir, or inject it directly into injection/recovery port. Turn off vacuum while filling slide holders.
	Reagent carry-over	Leave the vacuum running for at least one minute after tissue section chamber appears empty, then wash multiple times with appropriate wash buffer.
Inconsistent staining	Low antibody volume	Make sure that the antibody completely covers the tissue sample.
	Air bubbles	Use a 1 mL pipette tip and make sure that it is fully inserted into the injection/recovery port. Add the antibody very slowly, making sure that the liquid displaces any air bubbles left in the slide holder tissue section chamber. If bubbles form, use the pipette to withdraw the antibody (and bubbles) and re-inject. When dispensing is complete, remove the pipette tip slowly, so that air bubbles are not introduced into the tissue section chamber. Refer to "Tips for adding solution to slide holder" in the Staining Protocol section.
Low antibody volume recovery	Inefficient pipetting from the injection/recovery port	Use a 1 mL pipette tip and set the pipettor to the largest volume. Make sure that the pipette plunger is down when it is inserted into the injection/recovery port, then slowly released.
	Antibody leaked outside the tissue section chamber	Make sure that slide is correctly assembled in the slide holder, and that the four compression clips are completely engaged. Refer to Slide Holder Setup section.

Specifications

Dimensions

Base (length x width x height)	40.6 × 32.4 × 8.9 cm (16 × 12.75 × 3.5 in.)
Weight (approximate)	1.5 kg (3.3 lb)
IHC frame with cover	22.9 × 15.6 × 8.9 cm (9 × 6.125 × 3.5 in.)
IHC slide holder without slide	6.4 × 5.7 × 1.3 cm (2.5 × 2.25 × 0.5 in.)
IHC slide Holder with slide	7.6 x 5.7 x 1.3 cm (3 x 2.25 x 0.5 in.)

Materials of Construction

SNAP i.d.® 2.0 system	
Base and top	Acrylonitrile butadiene styrene (ABS)
Gaskets	Silicone
Tubing	Silicone
Tubing fittings	Polypropylene or acetal with ethylene propylene diene monomer (EPDM) or Buna-N seals
IHC Frame	
Top	Aluminum
Port plugs	Polycarbonate and Kraton® elastomer/ polystyrene
Valves/bushing	EPDM/nitrile
Incubation cover	ABS
IHC Slide Holder	
Slide holder	Polycarbonate
Gasket	Silicone
IHC Assembly Fixture	Aluminum

Chemical Compatibility

The SNAP i.d.® 2.0 Protein Detection System is compatible with aqueous solutions and dilute acids and bases. Do not expose to organic solvents. Some stains such as hematoxylin and eosin may permanently stain the system.

Storage

Store all components at room temperature.

Ordering Information

This section lists catalogue numbers for the SNAP i.d.® 2.0 system and associated products. See Technical Assistance section for contact information. You can purchase these products on-line at www.millipore.com/products.

Product Description	Cat. No.	Qty/Pk
Base System		
SNAP i.d.® 2.0 Base Base unit (1) Tubing assembly kit (1) Blot roller (1) Rolling pad (1) Wetting trays (2) Antibody collection trays (2) Quick-Start Guide (1)	SNAP2BASE	1
Components For Immunohistochemistry (IHC) Procedures		
SNAP i.d.® 2.0 Immunohistochemistry Frame	SNAP2FRIHC	1
SNAP i.d.® 2.0 IHC Slide Holder	SNAP2SH	24
Antibodies		
Primary and secondary antibodies	go to www.millipore.com/antibodies	
Components for Western Blotting Procedures		
SNAP i.d.® 2.0 MultiBlot Holding Frame MultiBlot frame with lid (1) MultiBlot holders (2)	SNAP2FRMB01	1
SNAP i.d.® 2.0 Mini Blot Holding Frame (single pack) Mini frame with lid (1) Mini blot holders (2)	SNAP2FRMN01	1
SNAP i.d.® 2.0 Mini Blot Holding Frames (double pack) Mini frame with lid (2) Mini blot holders (4)	SNAP2FRMN02	1
SNAP i.d.® 2.0 Midi Blot Holding Frame (single pack) Midi frame with lid (1) Midi blot holders (2)	SNAP2FRMD01	1
SNAP i.d.® 2.0 Midi Blot Holding Frames (double pack) Midi frame with lid (2) Midi blot holders (4)	SNAP2FRMD02	1

Ordering Information, continued

Product Description	Cat. No.	Qty/Pk
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
SNAP i.d.® 2.0 Antibody Collection Tray	SNAPABTR	20
SNAP i.d.® Blot Roller	SNAP2RL	1
Accessories		
Filter forceps, blunt end, stainless steel	XX6200006P	3
Vacuum filtering flask, 1 L	XX1004705	1
High Output Pump, 115 Volts, 60 Hz	WP6211560	1
High Output Pump, 100 Volts, 50/60 Hz	WP6210060	1
High Output Pump, 220 Volts, 50 Hz	WP6222050	1
Vacuum tubing, 6.4 mm ID x 3 m (1/4 in. ID x 10 ft)	MSVMHTS09	1
Millex®-FA_{50} filter unit, 1.0 µm, hydrophobic PTFE, 50 mm	SLFA05010	10

Conformance to Pressure Equipment Directive

The SNAP i.d.® 2.0 system does not fall within the scope of Pressure Equipment Directive 97/23/EC (PED), therefore, conformance to this directive is not applicable.

Technical Assistance

For more information, contact the office nearest you. In the U.S., call 1-800-221-1975. Outside the U.S., go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Standard 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).