Application Note

Antibody recovery and reuse in the SNAP i.d.® 2.0 immunodetection system

Introduction

Antibody reuse for Western blotting is a common practice for many researchers. While many antibodies lose potency with time or degrade even faster due to improper storage conditions, it is important to recognize the potential value of recovering the primary antibody for possible reuse in some experiments. The SNAP i.d.® 2.0 system is not only able to reduce the immunodetection processing time, but its flexibility lets you combine conditions used in the standard immunodetection protocol and also allows the collection of antibody for future reuse.

Here, we compare antibody recovery and reuse in the standard immunodetection protocol with the antibody recovery and reuse in SNAP i.d.® system using the extended protocol and the original SNAP i.d.® protocol.

Materials and Methods

Protein sample preparation

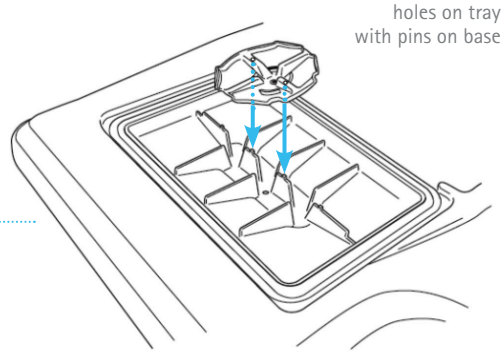
MCF-7 (ATCC® HTB-22™) breast cancer cell line and T47D P17 (ATCC® HTB-133™) ductal breast carcinoma cell line were grown to confluency. Total cell number was determined using the Scepter™ 2.0 cell counter (Cat. No. PHCC20060). The cells were washed with phosphate-buffered saline (PBS) and separated into two equal samples for lysis. Cells were lysed with 1 mL CytoBuster™ Protein Extraction Reagent (Cat. No. 1009-50mL) containing Benzonase® nuclease and rLysozyme™ reagent. The lysate was homogenized for few seconds with a handheld homogenizer and centrifuged at 15,000 x g for 10 minutes. The pellet was discarded and supernatant was saved for protein analysis. Total protein was quantitated using the Direct Detect™ IR-based quantitation system (Cat. No. DDHW000-10-WW).

Electrophoresis and blotting

Protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using commercially prepared, 1 mm thick, 4-12% gradient gels and MES running buffer. Gels were removed from the cassette and equilibrated for 10 min in transfer buffer (25 mM Tris, 192 mM glycine) supplemented with 10% methanol. After equilibration, the proteins were transferred to Immobilon®-P PVDF membrane (0.45 µm Cat. No. IPVH07850) using a semidry transfer system (10 Volts for 35 min). Blots were briefly rinsed in Milli-Q® water and were either allowed to air-dry or processed immediately.

Figure 1.
SNAP i.d.® 2.0 protocol, including antibody collection.

1. Assemble blot in blot holder/frame
2. Add blocking solution
3. Apply vacuum until completely dry
4. Turn vacuum off
5. Remove blot holder frame
6. Wipe any residual liquid at the bottom of the frame
7. Place antibody collection tray
8. Add primary antibody and incubate for 10 min or more
9. After incubation, turn vacuum on and wait for 1 min
10. Turn vacuum off and remove collection tray
11. Turn vacuum on for washing
12. Continue with the SNAP i.d.® protocol



Immunodetection and antibody recovery

Blots processed in the SNAP i.d.® 2.0 system were assembled according to the user guide. Once the assembled blot was placed in the SNAP i.d.® 2.0 system, blocking buffer (0.5% nonfat dry milk (NFDM) diluted in TBS-T (Tris-buffered saline with Tween® 20) was added and the vacuum immediately activated. The antibody collection tray was placed in the base (Figure 1), and the primary antibody, (anti-Type 2 protein serine/threonine phosphatase PP2A, Cat. No. 05-421) previously diluted according to table 1, was incubated for either 10 min or 1 hour depending on the method. To ensure optimal antibody recovery, the vacuum was kept on for at least 1 min, then the tray was removed, and the antibody was transferred to a suitable container for future reuse. Washes and secondary antibody incubation were performed according to the original SNAP i.d.® protocol (Table 1).

Considerations for Successful Antibody Recovery

1. To minimize antibody dilution, extend the vacuum time after the blocking step for a whole minute to make sure all the blocking solution has been removed.
2. Position the collection tray at the center of the base, aligning the holes of the tray with the pins at the base.
3. While collecting the antibody, turn vacuum on for a minute to ensure all the antibody has been transferred to the tray.
4. When processing two blots at the same time, it is recommended to collect only one side of the base at one time.
5. Blocking buffer is not optimal for antibody storage. It is recommended that recovered antibodies be reused within one or two days.

The breast cancer lysate blots were probed with monoclonal antibodies specific for PP2A (1:3,000) for the standard immunodetection protocol or the SNAP i.d.® extended protocol and 1:600 for the SNAP i.d.® original protocol. The antibodies were either freshly diluted in blocking solution or transferred to a clean tube and kept at 4 °C until reuse following the SNAP i.d.® system protocol. The blots were probed with HRP-conjugated secondary antibody (goat anti-mouse HRP, Cat. No. AP124P), diluted to,1:50,000 for the standard immunodetection protocol and 1:10,000 for both the SNAP i.d.® extended and original protocols.

Probed blots were incubated with Luminata™ Forte Western HRP substrate (Cat. No. WBLUF0100) and exposed to x-ray film.

Table 1.
Conditions used for the three different immunodetection methods analyzed. Note the difference in processing times between the 3 methods and the advantage of time savings offered by the SNAP i.d.® immunodetection methods.

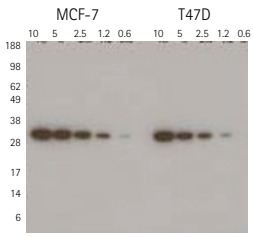
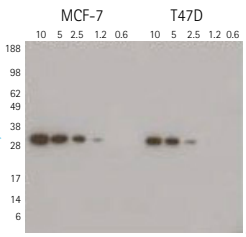
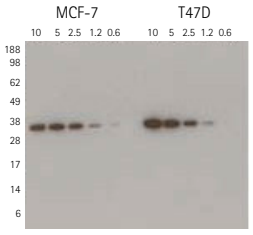
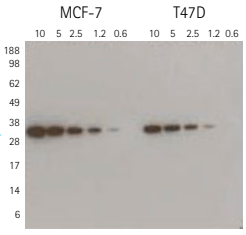
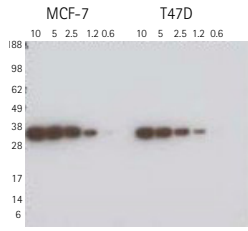
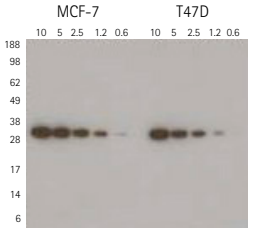
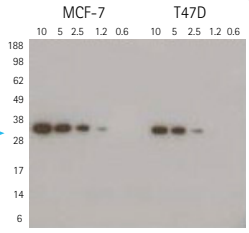
Step	Standard Method			SNAP i.d.® Extended Protocol			SNAP i.d.® Original Protocol		
	Volume	Conc.	Time	Volume	Conc.	Time	Volume	Conc.	Time
Blocking/NFDM	30 mL	0.5%	1 h	30 mL	0.5%	20 s	30 mL	0.5%	20 s
Primary Ab	10 mL	1:3,000	1 h	10 mL	1:3,000	1 h	5 mL	1:600	10 min
Wash	20 mL x 3	TBS-T	5 min each wash =15 min	30 mL x 4	TBS-T	20 s each wash = 80 s	30 mL x 4	TBS-T	20 s each wash = 80 s
Secondary Ab	10 mL	1:50,000	1 h	10 mL	1:10,000	10 min	5 mL	1:10,000	10 min
Wash	20 mL x 3	TBS-T	5 min each wash =15 min	30 mL x 4	TBS-T	20 s each wash =80 s	30 mL x 4	TBS-T	20 s each wash = 80 s
Total Processing Time			**3.5 h**			**< 1.5 h**			**< 30 min**

Results

To demonstrate the antibody collection feature of the SNAP i.d.® 2.0 system using the original protocol as well as in the extended protocol and its effect on antibody activity in subsequent Western blotting experiments, a set of identical blots were probed with anti-PP2A, either freshly diluted or previously used and recovered (Figure 2). A third set of identical blots was used as a control, following the standard immunodetection method. In the case of the extended protocol, the antibody was collected one more time and used for a third time, after an overnight incubation.

The average volume of antibody recovered in the SNAP i.d.® 2.0 system was 4.7 mL (94% of original antibody volume). All blots showed similar absolute and relative signal intensity, without appreciable nonspecific signal (background).

Figure 2.
Two-fold dilution series of breast cancer cell lysates (MCF-7 and T47D, 10 to 0.6 µg total protein) were subjected to SDS-PAGE and transferred to blotting membranes in seven identical blots using three different immunodetection protocols as described above. The seven blots were probed with anti-PP2A, freshly diluted, collected or recovered for a second time.

Method	Processing time	Blot processed with freshly diluted antibody	Blot processed with collected antibody	Processing time	Blot processed with antibody used for the third time
Standard/ Traditional Immunodetection Method	Blocking = 1 h Primary Ab =1 h Secondary Ab =1h 6 washes (5 min each) = ½ h **Total = 3.5 h**				
SNAP i.d.® Extended Protocol (Extended incubation of primary Ab only)	Blocking = 20 s Primary Ab = 1 h Secondary Ab =10 min 8 washes (20 s each) = 2 min 40 s **Total = 1 h 13 min**			Blocking = 20 s Primary Ab = Overnight Secondary Ab =10 min 8 washes (20 s each) = 2 min 40 s **Total = 14 hr 12 min**	
SNAP i.d.® Original Protocol	Blocking = 20 s Primary Ab = 10 min Secondary Ab =10 min 8 washes (20 s each) = 2 min 40 s **Total = 23 min**				

Conclusion

The SNAP i.d.® 2.0 Protein Detection System not only reduces Western blot processing time by as much as 80%, but it also conserves precious antibodies by providing a rapid and convenient method for the collection of primary antibodies for future reuse. Greater than 90% of the primary antibody volume can be recovered after the incubation step by following the recommended protocol. As demonstrated, the antibodies collected in the SNAP i.d.® 2.0 System can be used successfully in subsequent immunodetection with no reduction in blot quality, even in the extended protocol (1 h or overnight incubations).

SNAP i.d.® 2.0 System Ordering Information

Description	Qty/Pk	Catalogue No.
SNAP i.d.® 2.0 Base Includes: Base unit (1) Tubing assembly kit (1) Blot roller (1) Rolling pad (1) Wetting trays (2) Antibody collection trays (2) Quick-Start Guide (1)	1	SNAP2BASE
SNAP i.d.® 2.0 Mini Blot Holding Frame (single pack) Includes: Mini frame with lid (1) Mini blot holders (2)	1	SNAP2FRMN01
SNAP i.d.® 2.0 Mini Blot Holding Frames (double pack) Includes: Mini frame with lid (2) Mini blot holders (4)	2	SNAP2FRMN02
SNAP i.d.® 2.0 Midi Blot Holding Frame (single pack) Includes: Midi frame with lid (1) Midi blot holders (2)	1	SNAP2FRMD01
SNAP i.d.® 2.0 Midi Blot Holding Frames (double pack) Includes: Midi frame with lid (2) Midi blot holders (4)	2	SNAP2FRMD02
SNAP i.d.® 2.0 Mini Blot Holders	100	SNAP2BHMN0100
SNAP i.d.® 2.0 Midi Blot Holders	100	SNAP2BHMD0100
SNAP i.d.® 2.0 Antibody Collection Tray	20	SNAPABTR
SNAP i.d.® Blot Roller	1	SNAP2RL

Featured Products

Description	Catalogue No.
Immobilon®-P 0.45 µm PVDF membrane	IPVH07850
Anti-PP2A	05-421
Goat anti-mouse HRP	AP124P
Luminata™ Forte Western HRP substrate	WBLUF0100
Scepter™ 2.0 Handheld Automated Cell Counter	PHCC20060
CytoBuster™ Protein Extraction Reagent	71009-3
Direct Detect™ Spectrometer	DDHW00010-WW

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