

# Antibody Recovery and Reuse With the SNAP i.d.<sup>™</sup> Protein Detection System

## INTRODUCTION

Many researchers reuse antibodies in western blotting. While many antibodies lose potency with time or even degrade due to improper storage conditions, it is important to recognize the potential value of recovering the primary antibody for possible re-use in some experiments. Here we show how the SNAP i.d. Protein Detection System can be used to collect antibodies for future reuse. Since this system is vacuum-driven, following the protocol outlined below is critical for successful antibody recovery.

## RESULTS

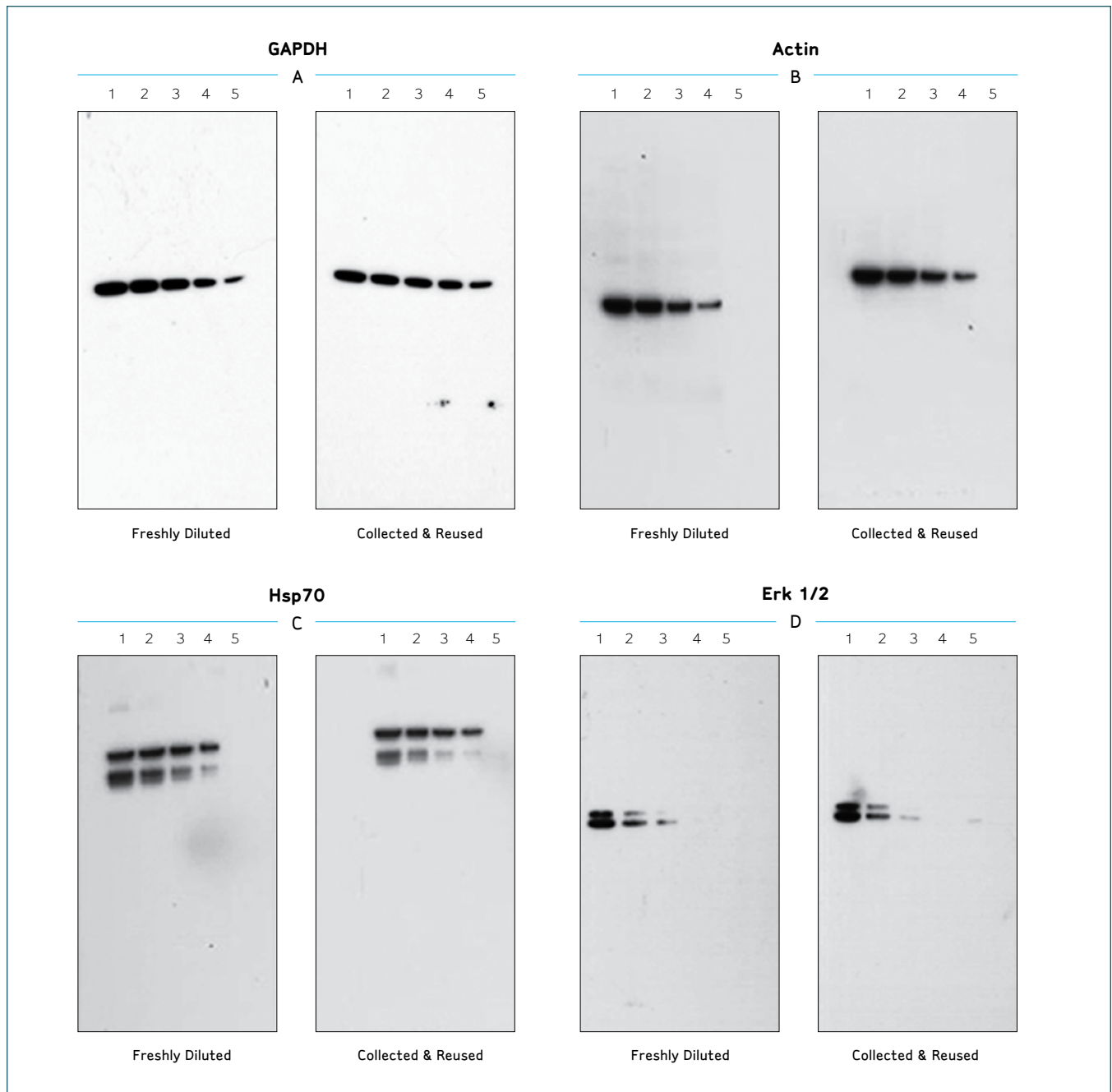
To demonstrate the antibody collection feature of the SNAP i.d. system and its effect on antibody activity in subsequent western blotting experiments, a set of identical blots was probed with several freshly diluted or recovered primary antibodies. The antibodies were collected using the SNAP i.d. system antibody collection trays as described under Materials and Methods and stored at 4 °C until reuse.

### Considerations for Successful Antibody Recovery

- Antibody recovery is only possible in single and double well blot holder.
- Position of the collection tray is critical for antibody recovery. For the single blot holder the tray should be positioned in the center of the manifold chamber. For the double well blot holder, the trays should be positioned with both trays' ID tabs facing each other (see Figure 2).
- Before the addition of antibody, the manifold lid must be properly closed. Proper sealing is essential to ensure that the antibody will be collected in the trays.
- In order to achieve maximum recovery, the system requires the addition of a small amount of buffer after the antibody incubation step. This is required to drive out antibody remaining in the blot holders. Although this step will result in a dilution of the collected antibody, it is essential for maximal recovery.

Figure 1 shows blots of rat liver or human epidermoid carcinoma (A431) cell lysates probed with the indicated antibodies using the SNAP i.d. system and double blot holders. On average there is less than 10% volumetric loss when antibodies are collected after immunodetection with the SNAP i.d. system (Table 1). The collected antibodies

were used to re-probe identically prepared blots with the SNAP i.d. system. Other than the difference in primary antibody (freshly diluted versus collected and reused) the blots were processed using the same protocol. As shown in Figure 1, despite the less than 100% recovery of the primary antibody and its subsequent dilution, there was



**Figure 1. Comparison of blots probed with fresh and re-used antibodies in double well blot holders using the SNAP i.d. system.**

A 2-fold dilution series of rat liver lysate (12 to 0.75  $\mu$ g) or A431 cell extract (20 to 2.5  $\mu$ g) were subjected to SDS-PAGE and transferred to blotting membranes as described under Materials and Methods. The rat liver lysate blots were probed with monoclonal antibodies specific for GAPDH (1:8,000), Hsp70 (1:8,000), and Actin (1:2,000); the A431 lysate blot was probed with an antibody (1:600 dilution) specific for Erk 1 (p44) which shows cross reactivity with Erk 2 (p42). The antibodies were either freshly diluted in TBST or collected and reused following the SNAP i.d. system protocol. The blots were probed with HRP-conjugated secondary antibodies, either goat anti-mouse at 1:10,000 (panels A – C) or goat anti-rabbit at 1:40,000 (panel D) in TBST.

only slight difference in band intensity and blot quality obtained with freshly diluted and collected and reused primary antibodies. In all cases, freshly prepared secondary antibody was used.

**Table 1. Antibody Recovery with the SNAP i.d. System.**

Blot Holder	Recovery (%)	No. of Expts.
Single Well	91 ± 6	8
Double Well	87 ± 8	16

The total percentage of antibody recovered after incubation and vacuum collection was determined by mass balance and is expressed as a percentage of challenge. Recovered primary antibody is suitable for subsequent western blotting.

## CONCLUSIONS

The SNAP i.d. Protein Detection System provides a rapid and convenient method for the collection of primary antibodies for future reuse. Greater than 90% of the primary antibody can be recovered after the incubation step by following the recommended protocol. As demonstrated herein, the collected antibodies can be used successfully for subsequent immunodetection using the SNAP i.d. system. As with standard immunodetection, not all antibodies will be amenable to their collection and reuse. Many antibodies are subject to degradation and either lose their potency or generate a higher background after their first use. These effects can be limited by proper storage and/or avoiding multiple freeze-thaw cycles. Despite the slight dilution of antibodies collected with the SNAP i.d. Protein Detection System, there is no reduction in blot quality in subsequent

experiments. The ability to collect and re-use primary antibodies coupled with the 30-minute immunodetection processing time, make the SNAP i.d. system ideal for most routine western blot analysis.

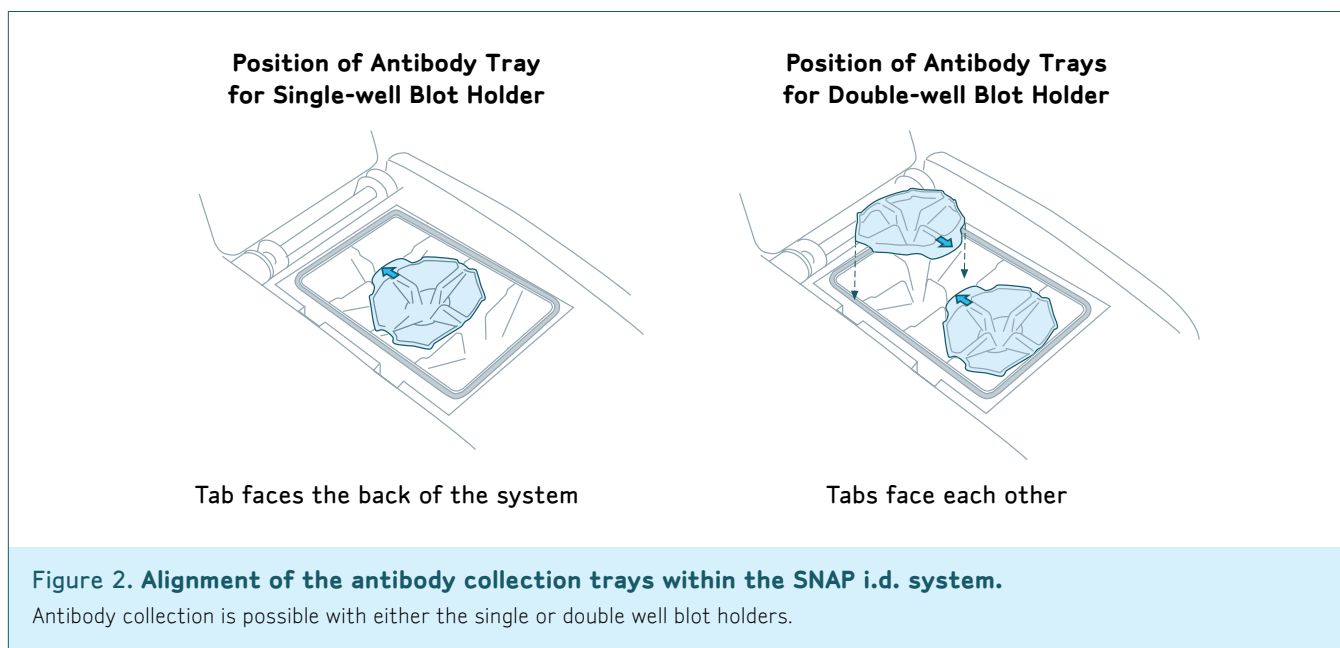
## MATERIALS AND METHODS

### Electrophoresis and Blotting

Solubilized protein samples were subjected to SDS-PAGE (4 – 12% gradient gels) using commercially prepared, 10-well, 1 mm thick minigels. After electrophoresis (typically 45 minutes at 200 V), the gels were removed from their cassette and equilibrated for 10 minutes at room temperature in transfer buffer (25 mM Tris, 192 mM glycine) supplemented with 10% methanol. The resolved proteins were transferred to Immobilon®-P polyvinylidene difluoride (0.45 µm PVDF) blotting membranes using a semi-dry transfer apparatus (BioRad) for 35 minutes at 10 V. After transfer, the blots were cut into 2 pieces and rinsed briefly in Milli-Q® water and allowed to either air dry for future use or assembled directly into the SNAP i.d. blot holder for immediate immunodetection.

### SNAP i.d. Immunodetection Protocol

Blots were processed as described in the SNAP i.d. Protein Detection System User Guide. Briefly, after the blot holders containing the blots were placed in the SNAP i.d. system, blocking buffer was added and the vacuum immediately activated. Primary antibodies diluted in blocking buffer were added to the blot holders and incubated for 10 minutes at room temperature. The vacuum was initiated and the blots were washed three times with TBST. After the vacuum was



**Figure 2. Alignment of the antibody collection trays within the SNAP i.d. system.**

Antibody collection is possible with either the single or double well blot holders.

turned off, the blots were incubated with a horseradish peroxidase (HRP) conjugated secondary antibody diluted in blocking buffer for an additional 10 minutes at room temperature. The vacuum was activated once again and the blots washed three additional times with TBST prior to visualization of the immunoreactive proteins.

### Antibody Collection

For antibody collection and reuse, the primary antibodies were recovered as described in Antibody Collection Tray User Guide. Briefly, the blots were blocked as described above. After removal of the blocking agent by vacuum, the blot holders were removed from the system, and the antibody collection trays were set in the chamber. The blot holders were then replaced, and the primary antibodies (diluted in blocking buffer) were added to the blot holders and incubated for 10 minutes at room temperature. Vacuum was then initiated for 10 – 20 seconds to ensure most of the antibody was emptied into the collection trays. After the vacuum was turned off, 1.0 mL of TBST wash buffer was added to each blot holder well. Vacuum was again

initiated for 10 – 20 seconds until all the TBST wash buffer was completely pulled through the blot holder into the collection tray. The vacuum was turned off, and blot holders and antibody collection trays were removed. The antibodies were transferred into 15-mL conical tubes and stored at 4 °C. The blot holders were placed back into the system for washing and incubation with secondary antibodies.

### Visualization of Immunoreactive Proteins

Probed blots were incubated with 5.0 mL of Immobilon® HRP Western Substrate for 5 minutes at room temperature. After patting the blots dry, they were exposed to x-ray film for 5 minutes and developed.

### Buffers

**TBST** – Tris-buffered saline solution (20 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20 surfactant).

**Blocking Buffer** – TBST supplemented with 0.5% non-fat dry milk.



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