Millipore®

User Guide

Immobilon® GO for Simple Immunodetection

This product is for research use only.

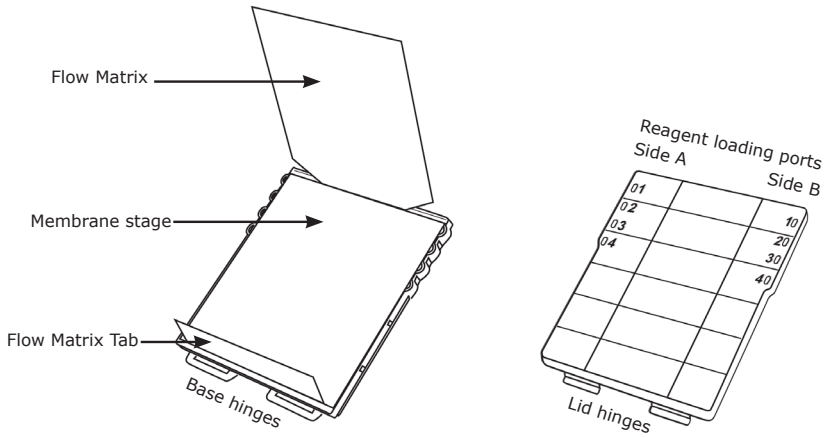
20347009, Rev. 10/18

Contents

Introduction

The Immobilon® GO device is a hands-free immunodetection device designed for walk away and stand-alone processing of Western blots. Each device can process one mini blot. The unique design uses continuous sequential fluid convection across a flow matrix to enable hands free antibody binding and membrane wash steps performed during immunodetection of western blots.

The Immobilon® GO device is intended for one-time use and does not require an external power or vacuum source for operation. Standard non-fat dry milk blot buffer is used throughout the Immobilon® GO set up to allow for easy transition from traditional western blotting protocols. Western blots are processed in as little as 3 hours; however, for greater convenience blots may remain in the Immobilon® GO device longer, even for overnight processing.



Immobilon® GO Base Assembly **Immobilon® GO Lid**

Figure 1: Immobilon® GO Overview

Materials required but not supplied

- Non-fat dry milk (NFDM)
- Tris buffered saline with Tween® 20 reagent (TBS-T) (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween® 20 reagent)
- Blot roller
- Forceps
- Incubation tray
- Primary and secondary antibodies

General guidelines

- Blotting membrane should not be larger than 8.5 cm x 8 cm. Immobilon® PSQ blotting membrane is not recommended for use with the Immobilon® GO device.
- The Immobilon® GO device requires a flat level surface for proper operation.
- Avoid indenting membrane when labeling.
- Once reagents have been added, do not move or open the Immobilon® GO device.
- Immunodetection is completed after 3 hours at room temperature.
- When performing immunodetection at 4 °C, overnight incubation (i.e., 16 hours) is required.
- Overnight incubation may also be performed at room temperature.

Preparation of 2% NFDM blot buffer

For each Immobilon® GO device to be used, prepare 25 mL of 2% NFDM blot buffer in TBS-T by completely dissolving 0.5 g of NFDM in 25 mL TBS-T. Mixture should be stirred for a minimum of 15 minutes prior to use.

Blotting Membrane Equilibration

If blotting membrane was previously dried, activate as described below.

- PVDF membrane: Activate by gently floating the membrane on a small volume of 100% methanol, ethanol or isopropanol for 10 seconds. Wash membrane well using deionized water to remove any residual alcohol.
- Nitrocellulose membrane: Wet membrane by gently floating on deionized water for 1 minute.

Place wet membrane, protein side up, into a dish slightly larger than the membrane. Distribute 10 mL of 2% NFDM blot buffer evenly across the membrane. Incubate without shaking for a minimum of 5 minutes.

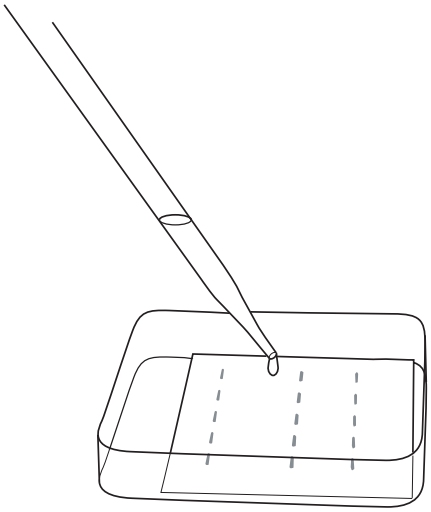


Figure 2. Blot equilibration

Primary Antibody Preparation

The Immobilon® GO device requires 1 mL of primary antibody diluted in 2% NFDM blot buffer.

Prepare primary antibody at 5-10 times higher concentration than the manufacturer's recommendation for standard Western blot immunodetection (e.g. prepare a 1:1,000 dilution if a 1:10,000 dilution is recommended). Because the volume is about 1/10 of that required for standard immunodetection, the amount of antibody required for each blot processed with the Immobilon® GO device is roughly equivalent. Further optimization may be required depending on the antibody and the detection reagent being used. Refer to Guidelines for Optimization on page 6 for additional detail.

Secondary Antibody Preparation

The Immobilon® GO device requires 1 mL of secondary antibody diluted in 2% NFDM blot buffer.

Dilution of the secondary antibody depends on the manufacturers recommended dilution as well as the sensitivity of the detection reagent. Typically, the secondary antibody is prepared 5-10 times more concentrated than manufacturer's recommendation for Western blot immunodetection (e.g., prepare a 1:1,000 dilution if a 1:10,000 dilution is recommended).

How to use the Immobilon® GO Device

1. Place the Immobilon® GO device on a level surface with loading ports positioned away from user.
2. Open the Immobilon® GO lid by gently pulling the purple snap lock loops located above the reagent loading ports over the grey locking tabs of the base. Fold lid up 90° and disengage from grey base hinge.

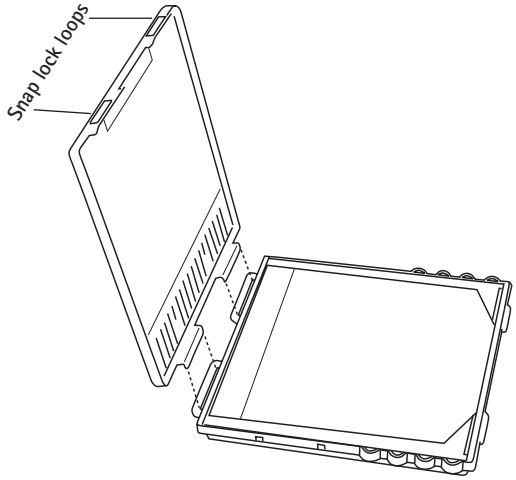


Figure 3. Opening Immobilon® GO

3. Fold back both the short and long flow matrices to expose the membrane stage.

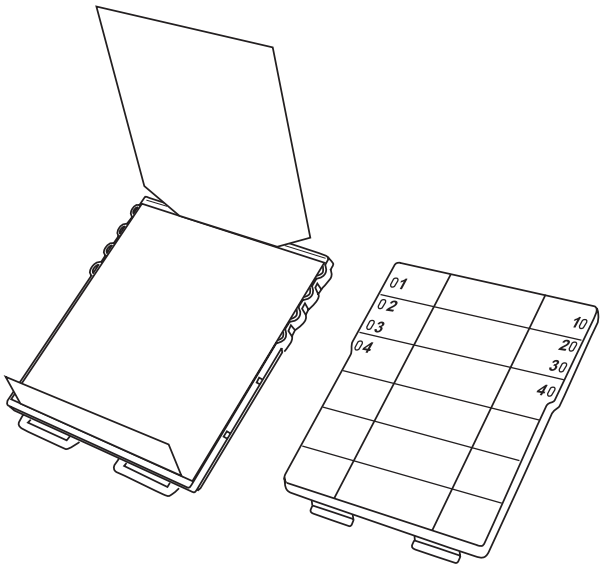


Figure 4. Folding back the flow matrix

4. Place the equilibrated membrane onto the Immobilon® GO stage with blotted proteins facing up and low molecular weight proteins facing the notched end of the flow matrix. Position blot so that it is centered side to side and the membrane is positioned 1 cm from the top edge of the stage.

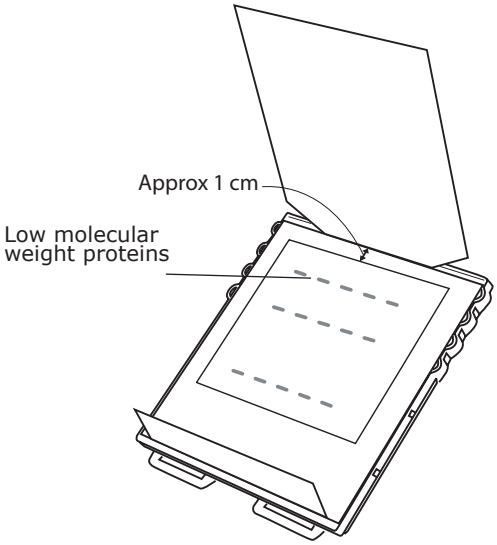


Figure 5. Blot placement

5. Bend the long flow matrix over the blotting membrane and gently hold it down with tweezers or a gloved hand. Starting at the widest part of the flow matrix near the loading port end, add 4 mL NFDM blot buffer in a serpentine pattern to provide even distribution across the flow matrix.

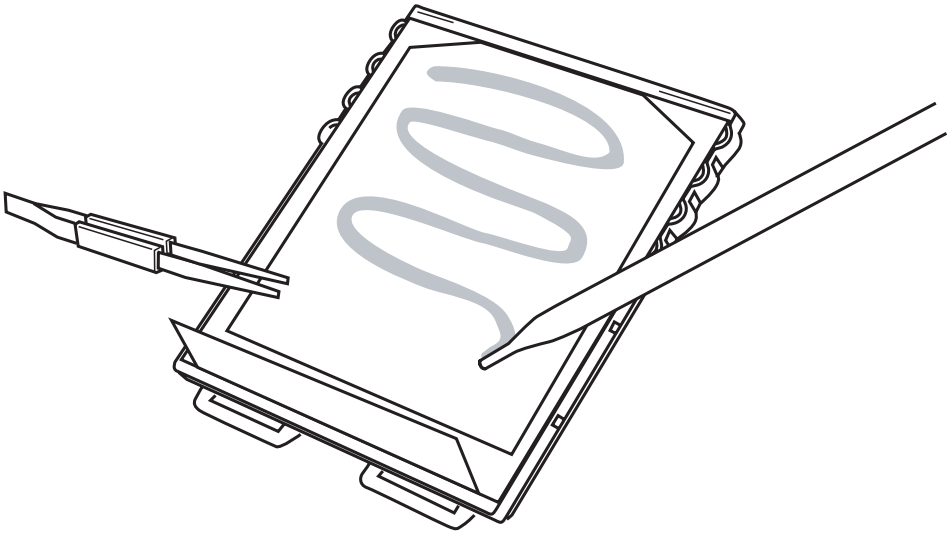


Figure 6. Flow matrix activation

6. Using the blot roller remove any air bubbles between the membrane, the stage and the flow matrix. Start blot roller at the loading port end and pull toward the short flow matrix tab. Assure blot roller is not placed onto the raised edge of the membrane stage.

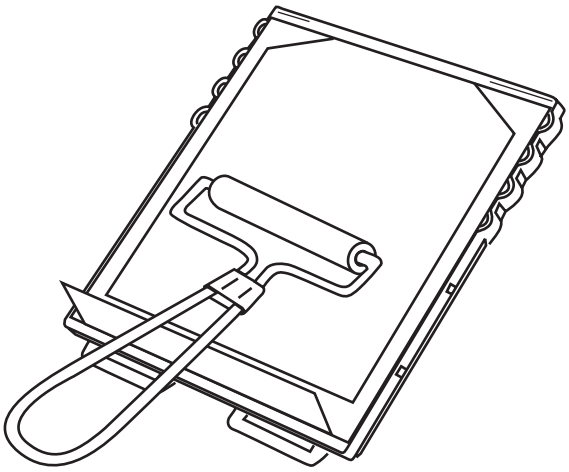


Figure 7. Remove air bubbles

7. Bend down the short flow matrix tab and remove any trapped air bubbles with the blot roller.
Note: Wash and dry the blot roller after each use.

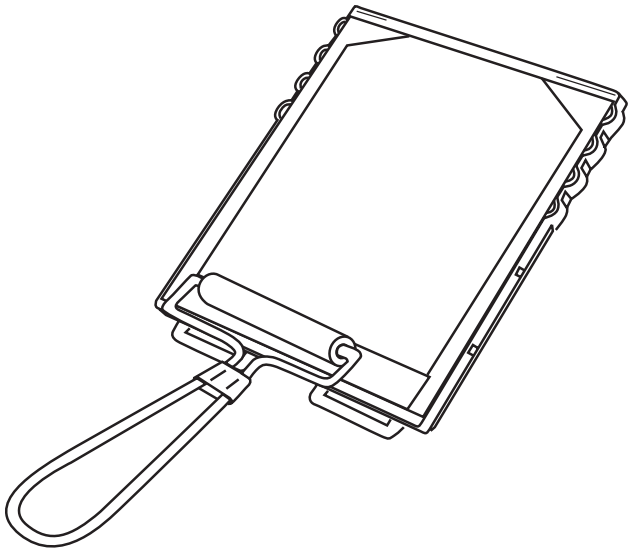


Figure 8. Prepare flow matrix tab

8. Close the Immobilon® GO device by inserting the lid hinges into the bases hinges and folding the lid onto the base. Push down on the lid as indicated below until the snap locks are secured. An audible click is heard when the Immobilon® GO device is properly closed.

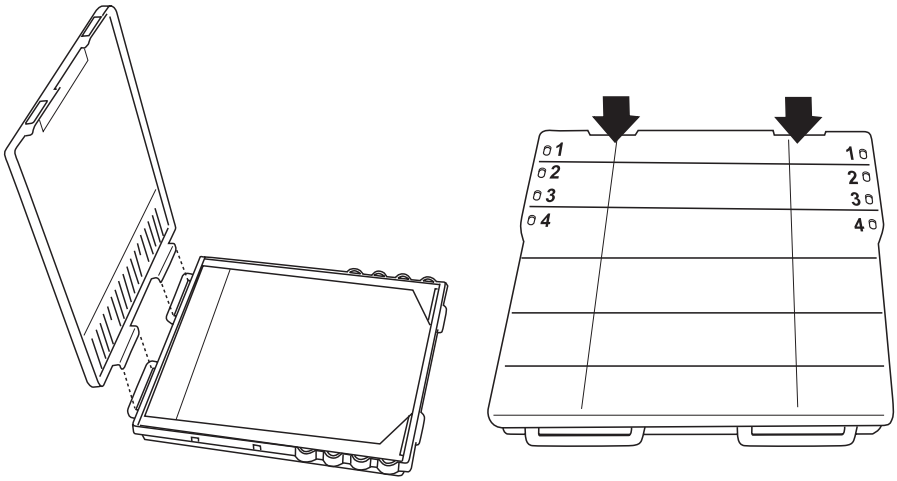


Figure 9. Closing the Immobilon® GO Device

Caution: Once reagents have been added, do not move the Immobilon® GO device.

9. Precondition the Immobilon® GO device by adding 0.5 mL of 2% NFDM blot buffer to each side of reagent loading port 1, followed by both sides of reagent port 2 and then 3. Add 3 mL of 2% NFDM blot buffer to each side of loading port 4 using a 5 mL serological pipette or 1 mL pipettor.
10. Add 0.5 mL of diluted primary antibody to each side of loading port 1.
11. Add 0.5 mL of diluted secondary antibody to each side of loading port 2.
12. Incubate device at room temperature for a minimum of 3 hours. Blots may be left in Immobilon® GO device overnight.
13. If using multiple Immobilon® GO devices, the lid may be labeled with experimental details.
14. Remove blot from the Immobilon® GO device and place it into a dish containing 50 mL TBS-T, wash for 5 minutes. Discard buffer. Repeat wash step.
15. Proceed with desired detection methodology.

Guidelines for Optimization

To obtain the best results, some antibody optimization will be required. Immunodetection results depend on the abundance of the target protein, antibody specificity and concentration, as well as the sensitivity of the detection method. Immunodetection results depend on the abundance of the target protein, antibody specificity, and concentration, as well as the sensitivity of the detection method to be used.

When working with known antibodies and chemiluminescent detection reagent, a good starting point for optimization with the Immobilon® GO device is to use the primary and secondary antibody 5-10 fold more concentrated than in standard immunodetection (i.e., if currently using 1:5,000 dilution, use 1:500 dilution with the Immobilon® GO device). Because the volume of antibody solution is about 10-fold less with the Immobilon® GO device than standard immunodetection methods, the amount of antibody required for each blot is roughly equivalent.

If starting with an unknown primary antibody, one needs to consider the sensitivity of the detection reagent to be used as well as the dilution recommended by the antibody manufacturer based on their model system. Tables 1 and 2 outline an example on how to optimize the primary as well as secondary antibody dilution.

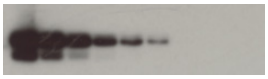
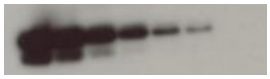
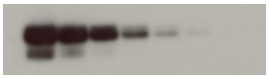
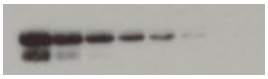
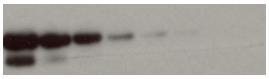
Manufacturer's recommended antibody dilution	Anti-histone (05-928): 1:2,000-1:20,000 Goat anti rabbit (HRP) (AP132P) 1:5,000-1:100,000		
Standard immunodetection antibody dilution	Primary: 1:2,000 Secondary: 1:10,000	Primary: 1:10,000 Secondary: 1:50,000	Primary: 1:50,000 Secondary: 1:250,000
			
Immobilon® GO antibody dilution	Primary: 1:200 Secondary: 1:2,000	Primary: 1:1,000 Secondary: 1:10,000	Primary: 1:5,000 Secondary: 1:50,000
			
Chemiluminescent Detection Reagent	Immobilon® Classico	Immobilon® Forte	Immobilon® ECL Ultra
Detection Reagent Sensitivity	Low	Mid	High

Table 1. Optimization of anti-histone and HRP conjugated goat anti rabbit antibody dilution based on manufacturer's recommendation and detection reagent sensitivity.

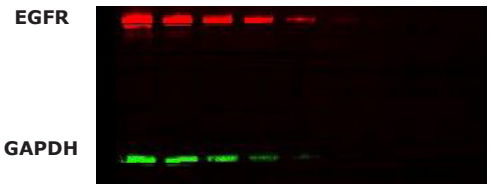
Catalog #	Host Species	Reactivity	Recommended* dilution for standard immunodetection	Typical Immobilon® GO antibody dilutions for Immobilon® Western HRP detection reagents		
				Classico	Forte	ECL Ultra
AP132P	Goat	Rabbit	1:5,000 - 1:100,000	1:2,000	1:10,000	1:50,000
AP124P	Goat	Mouse	1:5,000 - 1:100,000	1:1,000	1:5,000	1:25,000

*Based on product C of A

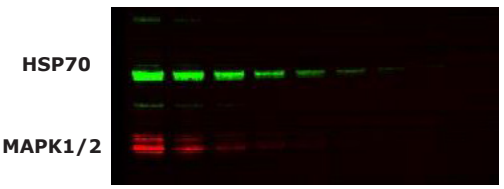
Table 2. Typical secondary antibody dilution based on Immobilon® Western HRP reagent sensitivity.

Fluorescent detection using the Immobilon® GO device

In addition to chemiluminescent detection, the Immobilon® GO device may also be used with fluorescently labeled secondary antibodies. As with chemiluminescent detection, single target and multiplexed fluorescent Western blot detection with the Immobilon® GO device requires some optimization. As a general rule, primary and secondary antibodies should be 5-10 times more concentrated than in standard fluorescent detection. Non-fat dry milk blot buffer is used for all antibody dilutions.



Detection of EGFR (06-847) and GAPDH (MAB374); Primary antibodies @ 1:100 dilution, fluorescently labeled secondary antibodies @ 1:100



Detection of HSP70 (SAB42007-14) and MAPK 1/2 (ABS44); Primary antibodies @ 1:100 dilution, fluorescently labeled secondary antibodies @ 1:100

Figure 10. Multiplex fluorescent detection with the Immobilon® GO Device

Troubleshooting

Observation	Cause	Recommendation
High background	Secondary antibody concentration too high	Increase dilution of secondary antibody
	Membrane was not equilibrated in blot buffer before using the Immobilon® GO device	Assure membrane is equilibrated for a minimum of 5 minutes; extend time if needed
	2% NFDM blot buffer incorrectly prepared	Review instruction for blot buffer preparation
	Membrane placed incorrectly	Membrane placement 1 cm from top edge of stage at loading port end of device
	Wrong detection reagent used	Antibody dilution needs to match detection reagent sensitivity
High background & strong signal	Primary and secondary antibody concentration too high	Reduce amount of primary and secondary antibody
Low or no signal of target protein	Primary antibody concentration too low	Increase primary antibody concentration (decrease dilution)
	Membrane placed incorrectly	Membrane placement 1 cm from top edge of stage at loading port end of device
	The Immobilon® GO device was moved after addition of reagents	Do not move the Immobilon® GO device once reagents have been added
	Immobilon® GO cartridge not properly activated	Follow the recommended protocol for reagent addition
	Primary and secondary antibody was added to wrong port	Review loading protocol
	Membrane not properly activated	Refer to manufacturer's protocol for membrane activation
	Target protein concentration too low	Load greater sample amount
	Protein did not properly transfer	Assure transfer conditions are optimized for protein of interest
Blot dried out during incubation	Wash buffer not added during activation step	Make sure all reagent addition instructions are followed
	Lid was not properly secured	A 'click' should be heard from both locking tabs

Storage Conditions

The Immobilon® GO device should be stored at room temperature, in a dry location out of direct sunlight.

Ordering Information

Immobilon® GO Device for Simple Immunodetection		
Description	**Qty/Pk**	**Catalogue Number**
Immobilon® GO Device	10 devices	IMGDV010
Immobilon® GO Device	2 devices	IMGDV002

Related Products for Western Blotting: Blotting Membranes		
Description	**Qty/Pk**	**Catalogue Number**
Immobilon® NOW Dispenser for 8.5 cm x 10 m rolls	1 dispenser	IMDISP
Immobilon®-E Membrane, PVDF, 0.45 µm, 8.5 cm x 10 m	1 roll	IEH85R
Immobilon®-E PVDF Transfer Membranes, 7 x 8.4 cm sheet	50 sheets	IEVH07850
Immobilon®-P Membrane, PVDF, 0.45 µm, 8.5 cm x 10 m	1 roll	IPVH85R
Immobilon®-P PVDF Transfer Membranes, 7 x 8.4 cm sheet	50 sheets	IPVH07850
Immobilon®-FL Membrane, PVDF, 0.45 µm, 8.5 cm x 10 m	50 sheets	IPFL85R
Immobilon® blotting filter paper, 7 × 8.4 cm sheet	100 sheets	IBFP0785C
Immobilon®-P Blotting Sandwich, 0.45 µm, 7 x 8.4 cm sheet	20 sandwiches	IPSN07852
Immobilon®-E Blotting Sandwich, 0.45 µm, 7 x 8.4 cm sheet	20 sandwiches	IESN07852

Related Products for Western Blotting: Chemiluminescent Detection Reagents		
Description	**Qty/Pk**	**Catalogue Number**
Immobilon® ECL Ultra Western HRP substrate	100 mL 500 mL	WBULS0100 WBULS0500
Immobilon® Western HRP substrate	50 mL 100 mL 500 mL	WBKLS0050 WBKLS0100 WBKLS0500
Immobilon® Forte Western HRP substrate	100 mL 500 mL	WBLUF0100 WBLUF0500
Immobilon® Crescendo Western HRP substrate	100 mL 500 mL	WBLUR0100 WBLUR0500
Immobilon® Classico Western HRP substrate	100 mL 500 mL	WBLUC0100 WBLUC0500

Related Products for Western Blotting: Membrane Stains and Stripping Reagents		
Description	**Qty/Pk**	**Catalogue Number**
Re-Blot™ Plus Strong Antibody Stripping solution, 10X	50 mL	2504
Reversible Protein Detection Kit for Membranes and Polyacrylamide Gels	1 kit	RPOB-1KT
Ponceau S solution, 0.1% (w/v) in 5% acetic acid	1 L	P7170

Secondary Antibodies		
Description	**Qty/Pk**	**Catalogue Number**
Goat Anti-Mouse IgG Antibody, Peroxidase Conjugated, H+L	2 mL	AP124P
Goat Anti-Rabbit IgG Antibody, Peroxidase Conjugated	2 ml	AP132P

Accessories		
Description	**Qty/Pk**	**Catalogue Number**
Blot Roller	1	SNAP2RL
Filter forceps, blunt end, stainless steel	3	XX6200006P

Reagents	
Description	**Catalogue Number**
Tris Base	93362
NaCl	71376
12N HCl	H1758
Tween® 20	P9416

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