148PR

G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Swift™ Western Blotting System

For Fast Track Western Blots

(Cat. # 786-158, 786-158S)

INTRODUCTION ... 3
KIT COMPONENTS ... 3
STORAGE CONDITIONS ... 3
IMPORTANT ... 4
ADDITIONAL MATERIALS REQUIRED ... 4
PREPARATION BEFORE USE ... 4
PROCEDURE ... 5
TROUBLESHOOTING ... 7
  NO SIGNAL ... 7
  WEAK SIGNAL ... 7
  HIGH BACKGROUND, EXCESSIVE OR NON-SPECIFIC SIGNAL ... 7
RELATED PRODUCTS ... 7

INTRODUCTION

The Swift™ Western Blotting System is a unique system that reduces the blocking and antibody incubations on Western blot membranes from >4 hours to <90 minutes. Using a combination of proprietary wash and diluent buffers and our highly sensitive femtoLUCENT™ chemiluminescence detection reagent, the Swift™ Western Blotting System generates comparable result to traditional Western blotting procedures and other commercial "fast" Western blotting kits. An added advantage is that Swift™ Western Blotting System is designed to be used with any combination of primary and secondary antibodies, unlike other commercial kits that limit researcher's to rabbit or mouse primary antibodies.

Swift™ Western Blotting System is compatible with wet and semi-dry transfer systems and with all automated transfer systems on the market. The system is suitable for 8 blots (8 x 10cm); the sample size is suitable for 2 blots.

KIT COMPONENTS

Description	Cat. # 786-158	Cat. # 786-158S
Swift™ Wash Buffer [10X]	125ml	30ml
Swift™ Western Diluent	125ml	30ml
femtoLUCENT™ Luminol Solution	20ml	10ml
femtoLUCENT™ Peroxide Solution	20ml	10ml

STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store at 4°C and use aseptic techniques when handling Swift™ Western Diluent. If stored and handled correctly the kit components are stable for up to 1 year.

IMPORTANT

- For improved protein transfer efficiency, we recommend our *Efficient*™ Western Transfer Buffer (Cat. # 786-019) or our High Molecular Weight Transfer Buffer (Cat. # 786-423) for the transfer of proteins >70kDa.
- To reduce the time for protein transfer, we recommend our Swift™ Transfer Pads (Cat. # 786-370 to 786-375). The Swift™ Transfer Pads can reduce transfer times by up to 50%, whilst preventing overheating. For more details, visit www.GBiosciences.com.
- All Swift™ reagents and other solutions should be at room temperature before applying to the membrane.

ADDITIONAL MATERIALS REQUIRED

- PVDF or nitrocellulose membrane with transferred protein
- Primary antibody
- Secondary antibody specific for primary antibody coupled to horseradish peroxidase (HRP)
- Orbital shaker
- X-ray film

PREPARATION BEFORE USE

1. ***1X Swift™ Wash Buffer:*** Prepare 150ml for each blot, dilute 15ml Swift™ Wash Buffer [10X] 1:10 by adding to 135ml DI water. Keep at room temperature until required.
2. ***Diluted Swift™ Western Diluent:*** Prepare 10ml for each blot by adding 5ml Swift™ Western Diluent to 5ml 1X Swift™ Wash Buffer. Store at room temperature until required to dilute and incubate the secondary antibody.
   NOTE: *The primary antibody is prepared in undiluted Swift™ Western Diluent.*
3. ***Preparation of Working femtoLUCENT™ Detection Solution:*** Allow the solutions to warm to room temperature before use. For each 8 x 10cm membrane, immediately prior to use, mix 2.5ml of femtoLUCENT™ Luminol Solution with 2.5ml femtoLUCENT™ Peroxide Solution.

PROCEDURE

__NOTE:__ Ensure all solutions are at room temperature before applying to the membrane

1. Remove the PVDF or nitrocellulose membrane from the wet or semi-dry transfer apparatus and quickly rinse the membrane twice in 10-30ml DI water to remove residual transfer buffer.
   __NOTE:__ If required, the membrane can be stained before this step to visualize proteins and check transfer efficiency. We recommend our Swift™ Membrane Stain (Cat. # 786-677), a reversible protein stain that is over 500X more sensitive than Ponceau-S staining.
2. Add 10ml undiluted Swift™ Western Diluent and place on an orbital shaker for ~30 seconds, ensuring both sides of membrane are uniformly coated in Swift™ Western Diluent.
3. Add the primary antibody of choice direct to the tray containing the Swift™ Western Diluent to a final concentration of 0.01-0.2µg/ml. Incubate at room temperature on an orbital shaker for 30 minutes.
   __NOTE:__ As with all Western blotting protocols, the concentration of primary antibody used will have to be optimized. In addition, the incubation times can be reduced to 10 minutes or increased to overnight depending on the avidity of the primary antibody and concentration of antigen on the membrane. Each antibody should be optimized for incubation times.
   We recommend using the same concentrations and incubations for antibodies already optimized for standard Western blotting procedures.
4. Remove the membrane from the tray and shake off excess antibody solution. Transfer the membrane to a fresh, clean and dry tray.
5. Add 0.01-0.2µg/ml secondary antibody conjugated to HRP to the 10ml Diluted Swift™ Western Diluent (Step 2, Preparation Before Use).
   __NOTE:__ As with all Western blotting protocols, the concentration of the secondary antibody used will have to be optimized.
6. Add 10ml Secondary Antibody in Diluted Swift™ Western Diluent (Step 3, Preparation Before Use). Incubate at room temperature for 15 minutes with shaking.
   __NOTE:__ The incubation times can be reduced to 10 minutes or increased to 1 hour depending on the avidity of the secondary antibody and concentration of antigen/primary antibody complexes on the membrane. Each antibody should be optimized for incubation times.
   We recommend using the same concentrations and incubations for antibodies already optimized for standard Western blotting procedures.
7. Remove the membrane from the tray, shake to remove excess solution and then place membrane edge on a paper towel to remove excess antibody solution by capillary action. Do not dab membrane with paper towel or allow to completely dry

as this will result in uneven development. Transfer the membrane to a fresh, clean and dry tray.

8. Wash the membrane for 3 x 5 minutes in 30ml 1X Swift™ Wash Buffer.
   NOTE: *With some combinations of antibodies a higher background may occur. To further reduce the background increase both the number and length of washes.*
9. Incubate the membrane in 5ml Working femtoLUCENT™ Detection Solution for 1-5 minute at room temperature with gentle shaking.
10. Drain the detection reagent and wrap the membrane in saran wrap or clear plastic wrap and expose to an autoradiography film.
    NOTE: *Do not wash or rinse the membrane after addition/removal of the working detection solution.*
11. The membrane can be redeveloped within1-2 days of initial development, provided that the detection reagents are removed from the membrane within 30-60 minutes of the first developing procedure. After each developing procedure, wash the membrane with 50ml TBS with 0.05% Tween® 20. Keep the membrane moist and at 4°C.

TROUBLESHOOTING

No Signal:

a. Protein was not transferred completely from gel to the membrane.
b. Protein is over transferred and passed through the membrane.
c. Primary antibody is not of higher titer or specificity of peroxidase labeled secondary antibody was not appropriate for primary antibody.
d. Use fresh detection reagent and detection buffer.

Weak Signal:

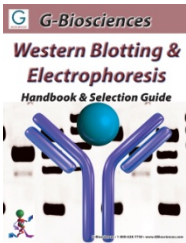
a. Solutions were not warmed to room temperature before addition.
b. Antibody concentration was too low or incubation time was too brief.
c. Not enough protein was loaded onto the gel or the primary antibody has low affinity for the target protein.

High background, Excessive or Non-Specific Signal:

a. Contamination with residual HRP. Ensure a clean tray is used where indicated.
b. Antibody was not diluted sufficiently or incubation times are excessive (adjust dilution & incubation time).
c. Blocking or washing procedures are inadequate (follow the recommended protocol).
d. The amount of antigenic protein loaded onto the gel is in excess.
e. The film was overexposed.

RELATED PRODUCTS

Download our Western Blotting & Protein Electrophoresis Handbook.



http://info.gbiosciences.com/complete-western-blot-handbook--selection-guide/

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 9/19/2012 CMH

www.GBiosciences.com