Western Blot for Protein Detection

Materials

Tris Base
Glycine
SDS
ddH_2O
Methanol
1 M Tris-HCl (pH 7.5)
NaCl
Tween-20
Skim milk
Bovine serum albumin (BSA)
PVDF membrane (0.2 μm)
Whatman filter paper
Transfer sponges
Mouse anti-His tag monoclonal antibody
Rabbit anti-DnaK antibody
HRP-conjugated goat anti-mouse IgG
Enhanced chemiluminescence (ECL) substrate
IRDye 680-conjugated goat anti-mouse IgG
IRDye 800-conjugated goat anti-rabbit IgG
Protein transfer apparatus
Shaker
Ice packs or ice-water bath
LI-COR Odyssey imaging system

Procedure

Buffer Preparation

Transfer Buffer

1. Prepare the following 1 L of 10× Transfer Buffer.

Reagent	**Amount**
Tris Base	30.25 g
Glycine	144.15 g
ddH_2O	To 1 L

2. Prepare 1× Transfer Buffer using the following reagents.

Reagent	**Volume**
10× Transfer Buffer	100 mL
ddH_2O	700 mL
Methanol	200 mL
10% SDS, if required for the transfer system	2.5 mL

TBS and TBST

1. Prepare the following 1 L of TBS.

Reagent	**Amount**
1 M Tris-HCl (pH 7.5)	20 mL
NaCl	8.8 g
H_2O	To 1 L

2. Add Tween-20 to TBS to a final concentration of 0.1% to prepare TBST.

Blocking Buffer

1. Prepare 1% (w/v) skim milk in TBST for Condition A.
2. Prepare 5% (w/v) BSA in TBST for Condition B.

Protein Transfer

PVDF Membrane Preparation

1. Cut the PVDF membrane to match the size of the gel.
2. Immerse the membrane in 100% methanol for a few seconds to activate it.
3. Briefly rinse the membrane with ddH_2O.

Transfer Stack Assembly

1. Assemble the transfer stack in the following order, ensuring the correct orientation.

Layer	**Material**
1	Transfer sponge
2	Whatman filter paper
3	Gel
4	PVDF membrane
5	Whatman filter paper
6	Transfer sponge

Transfer Conditions
1. Add 1× Transfer Buffer to the transfer tank.
2. Transfer the proteins at the following conditions.

Parameter	Condition
Transfer buffer	1× Transfer Buffer
Voltage	100 V
Transfer time	1 h
Operating mode	Constant voltage
Cooling	Ice-water bath

3. Check the transfer efficiency after the transfer is complete.

Western Blot Detection
Use Condition A or Condition B as specified for the experiment. Both conditions use TBST as the primary antibody diluent. Condition A uses HRP and ECL chemiluminescent detection, whereas Condition B uses infrared fluorescence detection with a LI-COR Odyssey imaging system.

Condition A HRP and ECL Detection

Membrane Washing
1. Remove the PVDF membrane from the transfer apparatus.
2. Rinse the membrane once with TBST to remove residual Transfer Buffer.

Membrane Blocking
1. Add approximately 25 mL of 1% skim milk in TBST.
2. Incubate at room temperature for 1 h with gentle shaking.

Primary Antibody Incubation
1. Remove the Blocking Buffer.
2. Prepare the primary antibody solution using the following conditions.

Parameter	Condition
Primary antibody	Mouse anti-His tag monoclonal antibody
Dilution	1:1000
Diluent	TBST
Solution volume	Approximately 5 mL
Temperature	4 °C
Incubation time	Overnight

3. Incubate the membrane in the primary antibody solution with gentle shaking.

Washing After Primary Antibody Incubation
1. Remove the primary antibody solution.
2. Wash the membrane with TBST three times for 10 min each.
3. Gently shake the membrane during each wash.

Secondary Antibody Incubation
1. Prepare the secondary antibody solution using the following conditions.

Parameter	**Condition**
Secondary antibody	HRP-conjugated goat anti-mouse IgG
Dilution	1:5000
Diluent	TBST
Solution volume	Approximately 10 mL
Temperature	Room temperature
Incubation time	50 min

2. Incubate the membrane in the secondary antibody solution.

Washing After Secondary Antibody Incubation
1. Wash the membrane after secondary antibody incubation.

Imaging
1. Image the membrane after the final wash to detect the target protein.

Condition B Infrared Fluorescence Detection

Membrane Washing
1. Remove the PVDF membrane from the transfer apparatus.
2. Rinse the membrane once with TBST to remove residual Transfer Buffer.

Membrane Blocking
1. Add sufficient 5% BSA in TBST to completely cover the membrane.
2. Incubate at room temperature for 1 h with gentle shaking.

Primary Antibody Incubation

1. Remove the Blocking Buffer.
2. Prepare the primary antibody solution using the following conditions.

Parameter	Condition
Primary antibody	Mouse anti-His-tag antibody and rabbit anti-DnaK antibody
Dilution	1:10,000 each
Diluent	TBST
Temperature	4 °C
Incubation time	Overnight

3. Incubate the membrane in the primary antibody solution with gentle shaking.

Washing After Primary Antibody Incubation

1. Remove the primary antibody solution.
2. Wash the membrane with TBST three times for 10 min each.
3. Gently shake the membrane during each wash.

Secondary Antibody Incubation

1. Prepare the secondary antibody solution using the following conditions.

Parameter	Condition
Secondary antibody	IRDye 680-conjugated goat anti-mouse IgG and IRDye 800-conjugated goat anti-rabbit IgG
Dilution	1:10,000 each
Diluent	TBST
Temperature	Room temperature
Incubation time	1 h

2. Incubate the membrane in the secondary antibody solution, protected from light.

Washing After Secondary Antibody Incubation

1. Wash the membrane after secondary antibody incubation.

Imaging

1. Image the membrane after the final wash using a LI-COR Odyssey infrared imaging system to detect the immunoreactive bands.