Western blotting

Supply and Reagents:

10XPBS (1L):

-80 g NaCl
-2 g KCL
-14.4 g Na_2HPO4 (dibasic, anhydrous)
-2.4 g KH_2PO_4 (monobasic anhydrous)
Adjust pH to 7.4, autoclave.

PBST: 100ml

10X PBS 100ml
Tween 20 1ml
dd water 900ml
Keep at room temperature

Transfer buffer (1L)

Glycine 14.4 g
Tris 3.0g
Methanol 200ml
Do not adjust pH by adding acid or bas, Keetp @ RT

Other:

Thick filter paper (Bio-Rad)
Immobilon-P (10X15cm)
Supersignal West Pico Chemiluminescent Sbustrate (Pierce #34077)
X-ray file (X-OMAT)
Tap
Timer
Radioatography cassette

Protocol:

1. Isolate protein, run SDS-PAGE gel.

2. Blotting

1. Equilibrate the gel in transfer buffer for 10min
2. PVDF membrane was put in methanol for 1min, then put in transfer buffer
3. Sandwich the gel and membrane

(from bottom to top: filter paper, membrane, gel, filter paper; depends)

4. Transfer the protein at 0.2 A, 30min, Do not exceed 25V with this instrument.
5. Wash the membrane with PBST 20ml, 5 min
6. Mark the marker protein band by pencil (optional)

(The membrane can be dry for 30mins, treated with methanol, and PBST before use)

3. Blocking: in 20ml

Blocking solution 50ml
Nonfat dry milk 2.5g
PBST 50ml
Transfer the membrane to blocking solution.
Gently agitated for 1h @ RT

4. **Reaction with antibody: in 10-15ml**

Antibody solution:
Anti-His HPR antibody (Invitrogen) 1ul
PBST 10-15ml
(if use primary antibody, dilution could be 1: 10000 or higher)

Discard blocking solution
Wash lightly by PBST 20ml, 5min, twice, with agitating
Transfer the membrane to a tray containing antibody solution
Gently agitate overnight at cold room (12h)

***If you don't use secondary antibody, skip to Step 7**.

5. **Wash** membrane with 20ml PBST, 3 times, 10 min each
6. **Add secondary antibody** (1:10000~1:20000 dilution in 15-20 ml PBST), agitate for 60 min at room temperature

7. **Wash** membrane with 20ml PBST, 3 times, 10 min each.
8. **Chemiluminescence, exposure, and detection** at dark room

    Hybridization bag
    Radioautography cassette
    X-Ray file
    SuperSignal West Pico Chemiluminescent Substrate (Pierce #34077)
    Tape
    Timer
    Palafile
    Micropipette
    Kim-wipe

    Turn on the light. Wear gloves.
    Place the membrane on parafilm.
    Prepare working solution (1:1 mixing) (0.5ml each)
    Add onto the membarne
    Incubate for 5 min
    Remove excess liquid with an absorbent tissue
    Place the membrane in hybridization bag
    Put the membrane in cassette
    Turn off light
    Exposure X-ray film in cassette for 60s, 1min or 2min(depending on signal strength)
    Develop the film.