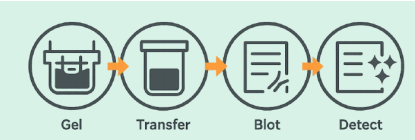
Instruction for Use (IFU)

Western Blot Consumable Combo, DS-WBCC-0925

This package provides all essential consumables required to perform a Western Blot experiment from protein extraction to chemiluminescent detection.



1. Required Equipment

(Not included in the combo, available separately)

Equipment	Model	Catalogue No.
Power Supply	PowerBasic 300	AP313001
Electrophoresis Cell	MiniPro4	AP320004
Blotting System	TBlotMini4	AP331004

2. Protein Extraction & Quantification

Product	Catalogue No.
RIPA Lysis Buffer	WSBL504A
Protease Inhibitor Cocktail Phosphotase Inhibitor Cocktail	WSBL630A WSBL636A
BCA Protein Assay Kit	WSBL1054A

Procedure

1. Lyse cells/tissues with RIPA buffer supplemented with one Protease Inhibitor Tablet.
2. Clarify lysates by centrifugation.
3. Quantify protein concentration using the BCA Protein Assay Kit (measure OD at 562 nm).

3. SDS-PAGE Gel Electrophoresis

Product	Catalogue No.
5X SDS Loading Buffer	WSBL530B
Gel Loading Tips	WSBS-200-GT
Tris-Gly Precast Protein Gel Bis-Tris Precast Protein Gel	WSBL1008A WSBL1398A
R3Charge Protein Ladder	DS1282100
Tris-Gly-SDS Running Buffer Tris-MOPS-SDS Electrophoresis Buffer	WSBL603C WSBL1685A

Dissolve Tris-Glycine-SDS / Tris-MOPS-SDS Electrophoresis powder in 1 Liter of RO water for each sachet

Procedure

1. Mix protein samples with 5X SDS Loading Buffer, heat at 95 °C for 5 min.
2. Assemble MiniPro4 electrophoresis cell (AP320004), fill with 1X running buffer.
3. Load samples and protein ladder with gel tips.
4. Run gel at 120 V (stacking) → 150 V (resolving) using PowerBasic 300 supply (AP313001).

4. Protein Transfer

Product	Catalogue No.
Blotting Paper	WSBS-TPB-01B
Nitrocellulose Membrane PVDF Transfer Membrane	WSTM-NC-XS-45-020 WSTM-PVDF-XS-45-020
Tris-Gly Transfer Buffer Western Rapid Transfer Buffer	WSBL626A WSBL1311B

Dissolve Tris-Glycine Transfer powder in 800 mL of RO water for each sachet. Note, to add another 200 mL of methanol before use.
Dissolve Western Rapid Transfer Buffer powder in 800 mL of RO water for each sachet. Note, to add another 200 mL of 95% ethanol before use.

Procedure

1. Equilibrate gel in transfer buffer.
2. Assemble transfer sandwich: sponge → blotting paper → gel → nitrocellulose → blotting paper → sponge.
3. Transfer using TBlotMini4 vertical blot system (AP331004) at 90 V for ~1 h.

5. Immunoblotting

Product	Catalogue No.
Lyophilized PBST Lyophilized 1X TBST	WSBL1684A-005 WSBL1683A-005
Western Blocking Buffer	WSBL535A
Antibody Stripping Buffer	WSBL1381A

Dissolve PBST powder in 1 L of RO water for each sachet.

Procedure

1. Block membrane with Western Blocking Buffer for 1 h at RT.
2. Incubate with primary antibody (diluted in PBST + blocking buffer) overnight at 4 °C.
3. Wash 3 × 5 min in 1X PBST.
4. Incubate with HRP-conjugated secondary antibody for 1 h.
5. Wash 3 × 10 min in PBST.
6. If re-probing, strip with Stripping Buffer before repeating antibody incubation.

6. Detection

Product	Catalogue No.
ECL Chemiluminescent Substrate	WSBL520A

Procedure

1. Prepare substrate according to kit instructions.
2. Apply evenly to membrane surface.
3. Detect with X-ray film or digital imaging system

7. Important Notice

This product package and associated reagents are intended FOR RESEARCH USE ONLY (RUO). Not for use in diagnostic procedures.

For technical questions or support, please contact:

- support@darwinscience.com
- +65 8945 9141
- 158 Kallang Way, Singapore 349245