Rapid Western Blotting Kit

Code	Description	Size
N790-KIT	**Rapid Western Blotting Kit-Mouse-SDT**	1 Kit (15 blots)
N791-KIT	**Rapid Western Blotting Kit-Rabbit-SDT**	1 Kit (15 blots)
1B1322-KIT	**Rapid Western Blotting Kit-Goat-SDT**	1 Kit (15 blots)
1B1324-KIT	**Rapid Western Blotting Kit-Rat-SDT**	1 Kit (15 blots)
N792-KIT	**Rapid Western Blotting Kit-Mouse-WT**	1 Kit (15 blots)
N793-KIT	**Rapid Western Blotting Kit-Rabbit- WT**	1 Kit (15 blots)
1B1323-KIT	**Rapid Western Blotting Kit-Goat- WT**	1 Kit (15 blots)
1B1325-KIT	**Rapid Western Blotting Kit-Rat- WT**	1 Kit (15 blots)
N868-KIT	**Rapid Western Blotting Kit-Sample**	1 Sample Kit (4 blots)

SDT-semi dry transfer
WT-wet transfer

General Information

VWR Life Science AMRESCO's Rapid Western Blotting Kit streamlines each step of the Western Blotting procedure to reduce blotting time to about an hour. These versatile kits include all reagents except for user supplied primary antibodies and chemiluminescent detection reagents. No expensive transfer equipment is needed. The kit directly reduces the time for transfer and blocking steps and combines the primary and secondary incubations into a single step for additional time savings. Times and features for each step includes:

- Transfer from gel to membrane with Rapid Transfer Buffer:
  - 10-20 minutes
  - Compatible with both wet and semi-dry apparatus
  - PVDF and nitrocellulose membranes
- Membrane blocking with RapidBlock™:

  - 5 minutes blocking time
  - Compatible with both PVDF and nitrocellulose membranes
- Combined primary and secondary antibody incubation:
  - Complete in 30-45 minutes
  - Available with either anti-mouse or anti-rabbit HRP conjugated secondary antibodies

All reagents (excepting secondary antibodies) are protein-free formulations that minimize cross-reactivity and enhance antigen availability. Rapid Western Blotting Kit may be used with either PVDF or nitrocellulose membranes.

Each Kit Includes

- 150 mL Rapid Transfer Buffer, 10X (N790, N791, 1B1322, 1B1324)
  OR
  1.5L Rapid Transfer Buffer (N792, N793, 1B1323, 1B1325)
- 15 mL Rapid Block ™ Solution, 10X
- 15mL Rapid Antibody Diluent, 10X
- 75mL Rapid Wash Solution, 20X
- 30ul Rapid Western Anti-(Mouse, Rabbit, Goat, Rat) HRP

Sample Kit (N868) Includes:

- 2 x 100mL Rapid Transfer Buffer, 10X
- 2mL Rapid Block ™ Solution, 10X
- 2mL Rapid Antibody Diluent, 10X
- 6mL Rapid Wash Solution, 20X
- 5ul Rapid Western Anti-Mouse HRP
- 5ul Rapid Western Anti-Rabbit HRP

Storage/Stability

All components of the Rapid Western Blotting Kit (**EXCEPT SECONDARY ANTIBODY**) are stable as concentrated or 1X solutions for 1 year at room temperature (18-26°C).

Rapid Western HRP-conjugated antibodies should be aliquoted and stored at -20°C for up to 1 year. Freeze-thaw cycles should be avoided. Antibodies are stable several weeks if kept undiluted at 2-8°C.

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Materials Required But Not Supplied

Deionized Water
Chemiluminescent Substrate

Protocol/Procedure:

Notes:

- Rapid Western Blotting Kit is suitable for use with either PVDF or nitrocellulose membranes
- As with any immunoblotting procedure, antibody concentrations may need to be optimized empirically.
- Rapid Western Blot Secondary Antibody should be diluted immediately before use.

1. Transfer to Membrane

a. Semi-dry Transfer Apparatus

i. Prepare a sufficient volume of 1X Rapid Transfer Buffer by diluting 1 part 10X Rapid Transfer Buffer into 9 parts distilled, deionized water.

ii.Gel preparation:

- Wash gels in dH20 for 5 minutes
- Equilibrate in 1X Rapid Transfer Buffer for 5 minutes.

iii. Membrane preparation:

- PVDF membranes: Prior to use, pre-wet in 100% methanol, rinse briefly in dH20, then equilibrate in 1X Rapid Transfer Buffer for a minimum of 5 minutes.
- Nitrocellulose membranes: Equilibrate in transfer buffer prior to use.

iv. Assemble gel sandwich and semi-dry transfer unit according to manufacturer's instructions.

v. Transfer at 25V for 10 minutes at room temperature.

b. Wet Transfer Apparatus

i. Prepare a sufficient volume of 1X Rapid Transfer Buffer by diluting 1 part 10X Rapid Transfer Buffer into 9 parts distilled, deionized water.

ii. Membrane preparation:

- PVDF membranes: Prior to use, pre-wet in 100% methanol, rinse briefly in dH2O, then equilibrate in 1X Rapid Transfer Buffer for a minimum of 5 minutes.
- Nitrocellulose membranes: Equilibrate in transfer buffer for a minimum of 5 minutes prior to use.

iii. Assemble gel sandwich and wet transfer unit according to manufacturer's instructions.

iv. Transfer at 75V for 20 minutes at room temperature.

2. Blocking

a. Prepare a sufficient volume of 1X RapidBlock™ by diluting 1 part 10X RapidBlock™ Solution into 9 parts distilled, deionized water.

b. Submerge membrane in 1X RapidBlock™ and incubate for 5 minutes at room temperature with agitation.

3. Antibody Incubation

a. Prepare a sufficient volume (typically 5-10 ml per blot) of 1X Rapid Antibody Diluent by diluting 1 part 10X Rapid Antibody Diluent into 9 parts distilled, deionized water.

b. Dilute primary antibody as recommended by the supplier into 1X Rapid Antibody Diluent.

c. Dilute Rapid Western Secondary Antibody, HRP conjugated (N801-30UL, N802-30UL, 1B1311-30UL, or 1B1312-30UL into 1X Rapid Antibody Diluent solution containing primary antibody.

Example

1X Rapid Blot Antibody Diluet	Primary Antibody	Rapid Blot Secondary Antibody, HRP Conjugated
10ml	Supplier's Recommendation	2ul

d. Incubate the blot in antibody solution for 30-45 minutes at room temperature with agitation.

4. Washing

a. Prepare a sufficient volume of 1X Rapid Wash Solution by diluting 1 part 10X Rapid Wash Solution into 9 parts distilled, deionized water.

b. Wash the blot three times for 5 minutes each in 20ml 1X Rapid Wash Solution.

5. Detection

Visualize bands with enhanced chemiluminescent substrates for HRP linked reporter enzymes, such as AMRESCO's VisiGlo™ HRP Plus (N219-KIT).

Frequently Asked Questions

Question	Answer
Why is the signal on my blot weak?	1. Primary antibody binds to the antigen with low affinity. Increase the concentration of the primary antibody or increase incubation time. 2. Insufficient protein loaded on the gel. 3. Insufficient transfer of protein to the blotting membrane. Optimize transfer conditions and assess efficiency of PonceauS or ProAct™ Membrane Stain
Why is the background high on my blot?	1. Primary antibody concentration is too high. Reduce the amount of antibody used or the antibody incubation period. 2. Inadequate blocking of the membrane. Increase blocking time. 3. Methanol was used in the transfer buffer. Use only Rapid Transfer Buffer supplied with the kit or another methanol-free transfer buffer. 4. Insufficient washing. Increase the number and/or length of wash steps.
Why are there white bands on a black background on my blot?	Negative staining indicates antibody concentration is too high. Optimize antibody concentration and incubation time.
Can the Rapid Wester Kits be used with Phospho-specific antibodies?	Yes.
Can I use a different transfer method in combination with the blotting kit?	If using a transfer buffer other than the Rapid Transfer Buffer, 10X supplied with the kit, you must use a methanol-free replacement. Methanol in the transfer buffer increases background on the blot.
Can I use Rapid Transfer Buffer with the iBlot® Dry Blotting System or other similar system?	Rapid Transfer Buffer is not compatible with devices, such as the iBlot® Dry Blotting System.

Directions for Use

For Technical Support
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