



Rapid Western Blotting Kit

<u>Code</u>	<u>Description</u>	<u>Size</u>
N790-KIT	Rapid Western Blotting Kit-Mouse-SDT (<i>semi-dry transfer</i>)	1 Kit
N791-KIT	Rapid Western Blotting Kit-Rabbit-SDT (<i>semi-dry transfer</i>)	1 Kit
N792-KIT	Rapid Western Blotting Kit-Mouse-WT (<i>wet transfer</i>)	1 Kit
N793-KIT	Rapid Western Blotting Kit-Rabbit-WT (<i>wet transfer</i>)	1 Kit

Each kit includes

Rapid Transfer Buffer, 10X, 150 ml

RapidBlock™ Solution, 10X, 15 ml

Rapid Antibody Diluent, 10X, 15 ml

Rapid Wash Solution, 20X, 75 ml

Rapid Western Anti-Mouse HRP or Rapid Western Anti-Rabbit HRP, 30ul

Sufficient material for 15 blots

N868-KIT RAPID WESTERN BLOTTING KIT – SAMPLE

Kit includes

Rapid Transfer Buffer, 10X, 2 x100 ml

RapidBlock™ Solution, 10X, 2 ml

Rapid Antibody Diluent, 10X, 2 ml

Rapid Wash Solution, 20X, 6 ml

Rapid Western Anti-Mouse HRP, 5 µl

Rapid Western Anti-Rabbit HRP, 5 µl

General Information:

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 include:

- 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.

Storage/Stability:

All components of Rapid Western Blotting Kit (**EXCEPT SECONDARY ANTIBODY**) are stable as concentrated or 1x solutions for 1 year at room temperature (18-25° C).

Rapid Western Anti-Mouse HRP or Rapid Western Anti-Rabbit HRP: Extended Storage: Contents should be aliquoted and stored at -20° C for up to 1 year. Freeze-thaw cycles should be avoided.

Undiluted solution: Stable for several weeks at 2-8° C. Dilute immediately prior to use.

Application Disclaimer

For Research Use Only. Not for Therapeutic or Diagnostic Use.

Protocol:

Reagents not included:

Deionized water

Chemiluminescent detection reagents

 **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 dH₂O 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 dH₂O, 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 Anti-Mouse HRP or Rapid Western Anti-Rabbit HRP into 1X Rapid Antibody Diluent solution containing primary antibody.

Example:

1X Rapid Blot Antibody Diluent	Primary Antibody	Rapid Blot Secondary Antibody, HRP Conjugated (anti-mouse or anti-rabbit)
10 ml	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).

Related Products*Electrophoresis*

M317 Fluorescent Sprint NEXT GEL™,
10% Solution

M318 Fluorescent Sprint NEXT GEL™,
12.5% Solution

Membrane stains

M282 Proact™ Membrane Stain

K793 Ponceau S Stain

Chemiluminescent Substrates

N218 VisiGlo™ Chemiluminescent
Substrate

N219 VisiGlo™ Plus Chemiluminescent
Substrate

Stripping Buffer

N552 Gentle Review™ Stripping Buffer

X-ray film background reducer

N656 UnDo™ X-Ray Film Background
Reducer

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