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Instructions for ImmunoBooster™ Western blot Assay

Cat. # RWA-500 500 ml

Introduction

The BIOWORLD Consulting Laboratories ImmunoBooster™ Rapid Western Blot buffer (**Patent Pending**) contains optimized reagents that shorten the time to perform a typical Western blot from 2-4 hours to ~20 minutes. The buffers can be used with mouse, rabbit or human antibody of polyclonal or monoclonal origin and any secondary antibody labeled with HRP, alk. Phosphatase, biotin, or with any type of fluorescence labels.
The protocol requires minimal hands-on time and yields results comparable or better than the classic Western blotting.

Important Product Information

- For optimal results, use a shaking platform during incubation steps.
- Use a tray with the size of the membrane to completely cover the membrane with the reagents.
- Do not handle membrane with ungloved hands. Always wear gloves or use clean forceps to handle the blot.
- The stability of primary antibodies diluted in the Rapid Western Antibody Diluent varies. For best results, dilute the antibody in the working solution no more than 24 hours before use.
- It is recommended as good laboratory practice to use gloves and personal protective gears especially as some serum samples if used in Western blotting may be infectious.
- The diluted antibody can be saved and reused for a second incubation of another membrane, if the solution is stored at +4^{0}C for a maximum of three days.

New one step Western blot protocol included see page 5.

If monoclonal antibody is used for the western blot it is possible to use one step incubation with the primary and secondary antibody simultaneously. It will save you both time and extends the reagent to be used for 50 western blots.

Material Included

- **ImmunoBooster ™ Rapid Western Antibody Diluent**
  Store at room temperature (15-30°C)
- **Rapid Western Blocking solution (5X concentrate)**
  Store at +4°C
- **10% Tween 20 solution**
  Store at room temperature (15-30^{0}C)

Sufficient material to perform 25 western blots (8 x 10 cm) or 50 western blots (one step blot)

Additional Materials Required

- Membrane with transferred protein. Use any protocol to separate proteins by electrophoresis and transfer them to a nitrocellulose or PVDF membrane.
- Primary Antibody: Choose a mouse, rabbit or human antibody that is specific to the target protein(s). The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane. However, in our experience the same dilution which was used with the regular western blotting works well. *In some cases, the primary antibody can be diluted 2 to 8 fold more with comparable results to the regular western blot (**Saves antibody**).*
- PBS or TBS
- PBS or TBS containing 0.05% Tween-20
- Shaker platform for agitation of membrane during incubations.

Material Preparation for two step Western blot

Rapid Western Blocking Solution **Working solution**	Mix 2 ml Rapid Western Blocking Solution with 8 ml TBS or PBS. Add 100 microliters of 10% Tween 20 solution and mix (Adequate for blocking one 8 × 10 cm blot)
Primary antibody dilution	Dilute the primary antibody with **Rapid Western Antibody Diluent** to a concentration ranging from 0.2 to2 µg/ml or what has been established in previous western assays. *It is possible that lower antibody concentration can be used than with the traditional western.* Use ~0.125 ml of antibody per cm^2 of membrane (e.g., 10 ml per 8 × 10 cm blot). For best results, prepare working dilution no more than 2 hours before use.
Secondary antibody dilution	Dilute the secondary antibody with the **Rapid Western Antibody Diluent** according to recommendation from the supplier (Generally 1/5,000 to 1/10,000 dilution) Use ~0.125 ml of antibody per cm^2 of membrane (e.g., 10 ml per 8 × 10 cm blot). For best results, prepare working dilution no more than 2 hours before use.

Procedure for Western Blotting (8 x10 cm blot)

BLOCKING

1. Remove blot from the transfer apparatus and place in a clean incubation tray (~10 x10 cm).
2. Briefly rinse blot in PBS or TBS.
3. Add 10 ml of the diluted (working solution) Rapid Western Blocking Solution to the blot and incubate for 2 minutes at room temperature with shaking.
4. Remove the Rapid Western Blocking Solution from the blot.

PRIMARY ANTIBODY

5. Immediately add 10 ml of the diluted Primary Antibody to the blot and incubate for 10 minutes at room temperature with shaking (incubation time can be extended to no more than 15 minutes to increase sensitivity).

WASH

6. Remove the Primary Antibody Solution from the blot and add 10 ml of PBS or TBS containing 0.1% Tween 20 and 1 ml of Rapid Western Blocking solutions (5X concentrate).
7. Incubate on shaker for 1 minute.
8. Remove the rinsing solution (if polyclonal antibody was used repeat the wash once).

SECONDARY ANTIBODY

9. Immediately add 10 ml of the diluted Secondary Antibody to the blot and incubate for 5 minutes at room temperature with shaking (incubation time can be extended to no more than 10 minutes to increase sensitivity).
10. Remove the Secondary Antibody Solution from the blot

FINAL WASH AND DEVELOPMENT

11. Wash membrane by adding 10 ml of PBS or TBS with 0.1% Tween 20 to the blot, and shake it for 2 minutes.
12. Remove the washing solution from the blot.
13. Wash membrane by adding 10 ml TBS or PBS to the blot, and shake it for 2 minutes
14. Repeat this wash once. (*For polyclonal antibodies used as primary antibody, it is recommended that the washing step should be repeated two more times to eliminate background using PBS or TBS containing 0.05% Tween-20*).
15. Remove blot, place it in a clean incubation tray, and develop with your detection reagent of choice as you did it with your regular Western blot.

Troubleshooting

Problem	Possible Cause	Solution
High background	Incubation tray is contaminated with HRP or alk.phosphatase	Use a clean incubation tray after every step of the procedure
	Insufficient washing	Use a minimum of 10 ml of Wash Buffer for each wash
		Add an additional wash cycle for a total of three washes
	Overexposed film	Decrease exposure time
	Omitted the blocking step	Block the membrane in the diluted Rapid Western Blocking Solution.
Weak signal	Used insufficient quantities of antigen or primary antibody	Strip and re-probe blot using a higher concentration of antibodies
		Load higher concentrations of sample onto the gel
	Inefficient protein transfer	Optimize transfer conditions
Strong signal but background bands are also visible	Too much antibody Because the buffer increases sensitivity, it also enhances cross-reactive epitope reactions.	Optimize concentration of the primary antibody. Use lower concentration of antibody.
	To much protein loaded on the gel	Load less amount of protein on the gel.

Question or comments:

Send email to **tech@bioworldantibodies.com**

Protocol to download:

www.bioworldantibodies.com/info/WESTERN_BLOT.pdf

Material Preparation for One Step Western blot

Important Information

One step western blot only works with monoclonal primary antibodies; do not use it with polyclonal primary antibodies. The secondary antibody must be **anti-IgG Fc** it cannot be anti-IgG H&L (anti-IgG H&L will block the antigen binding site).

Rapid Western Blocking Solution **Working solution**	Mix 2 ml Rapid Western Blocking Solution with 8 ml TBS or PBS. Add 100 microliters of 10% Tween 20 solution and mix (Adequate for blocking one 8 × 10 cm blot)
Primary antibody dilution	Dilute the primary antibody with **Rapid Western Antibody Diluent** to a concentration ranging from 0.2 to2 µg/ml or what has been established in previous western assays. *It is possible that lower antibody concentration can be used than with the traditional western.* Use ~0.125 ml of antibody per cm^2 of membrane (e.g., 10 ml per 8 × 10 cm blot). For best results, prepare working dilution no more than 2 hours before use.
Secondary antibody dilution	Dilute your secondary antibody **(anti-IgG Fc)** to1/5,000 or 1/10,000 in your diluted primary antibody solution. Mix and it is ready to use.

Procedure for One Step Western Blotting (8 x10 cm blot)

BLOCKING

1. Remove blot from the transfer apparatus and place in a clean incubation tray (~10 x10 cm).
2. Briefly rinse blot in PBS or TBS.
3. Add 10 ml of the diluted (working solution) Rapid Western Blocking Solution to the blot and incubate for 2 minutes at room temperature with shaking.
4. Remove the Rapid Western Blocking Solution from the blot.

PRIMARY AND SECONDARY ANTIBODY INCUBATION

5. Immediately add 10 ml of the diluted Primary Antibody and Secondary Antibody mix to the blot and incubate for 10 minutes at room temperature with shaking (incubation time can be extended to no more than 15 minutes to increase sensitivity).
6. Remove the Primary and Secondary Antibody Solution from the blot and add 10 ml of PBS or TBS containing 0.1% Tween 20.
7. Incubate on shaker for 2 minute.

FINAL WASH AND DEVELOPMENT

8. Wash membrane by adding 10 ml of PBS or TBS with 0.1% Tween 20 to the blot, and shake it for 2 minutes.
9. Remove the washing solution from the blot.
10. Wash membrane by adding 10 ml TBS or PBS to the blot, and shake it for 2 minutes
11. Remove blot, place it in a clean incubation tray, and develop with your detection reagent of choice as you did it with your regular Western blot.