Cyrusbioscience

ReadyBlot™ Fast Western Transfer Buffer, 10x

Cat. Number: IDWW13

Benefit

Utilizes proprietary formulation that drastically increases transfer speed while maintaining protein integrity with reduced heat generating buffer that prevents protein modification or degradation during transfer. ReadyBlot™ allows researchers to complete electroblot procedure in less than 10 minutes.

- High speed transfer: < 15 min compared to traditional Western transfer of 90 min to 4 hr
- Convenience: use existing equipment

Transfer Efficiency: 100% complete protein (6-240kD) transfer

- Ease of use: Intuitive easy-to-follow
- Compatibility: with any gel chemistry, electroblotting system, or membrane types

Protocol

ReadyBlot™ blotting buffer is a proprietary formulation that drastically increases the transfer speed without the excessive heat generation that can affect proteins. ReadyBlot™ is available liquid or pre-wet pad formats and is compatible with current Western blotting protocols. This buffer is also compatible with any membrane (PVDF, Nitrocellulose, Nylon, etc…).

1. Prepare the working solution

   i. Pour 100 ml 10x ReadyBlot™ stock in a one liter graduated cylinder.

   ii. Add 200 ml Methanol or Ethanol (95%).

   iii. Add 700 ml deionized or distilled water. Mix gently.

2. To prepare a Wet Transfer (protocol for one Western transfer).

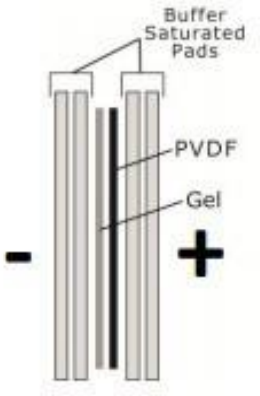
   i. Soak 4 pieces of thick blotting paper and one transfer sponge in the buffer.

   ii. For PVDF briefly soak the membrane in methanol or ethanol.

   iii. Transfer the membrane in the Fast Western Transfer Buffer.

   iv. Remove the gel from the cassette and let in soak about 2 min in distilled water.

   v. Prepare the electro-blotting sandwich (see figure to the right).



      i). Open the blot cassette, Cathode (-) electrode side towards you.

      ii). Add two layers of well soaked thick blotting paper (prevent bubbles).

      iii). Place the gel on the blotting paper, wet it with some blotting buffer.

      iv). Add the membrane and remove all bubbles, wet it with some extra buffer.

      v). Add two layers of well soaked thick blotting paper (prevent bubbles).

      vi). Add one wet sponge and press together while closing the support grid of the cassette.

      vii). Place the sandwich-cassette vertically in the tank.

3. To prepare a Semi-dry Transfer

i. Soak 4 pieces of thick blotting paper and four transfer sponges in the buffer 1 to 4 blots are done in Invitrogen XCell II™ Blot Module. Reduce the number of sponges accordingly.

ii. For PVDF briefly soak the membrane in methanol or ethanol.

iii. Transfer the membrane in the Fast Western Transfer Buffer.

iv. Remove the gel from the cassette and let in soak about 2 min in distilled water.

v. Prepare the electroblotting sandwich (see figure).

i). Open the blot module, the cathode (–) core in front of you.

For Invitrogen, the cathode core is the deeper of the 2 cores.

ii). Add two layers of well soaked sponges (prevent bubbles).

iii). Add two layers of well soaked thick blotting paper (prevent bubbles).

iv). Place the gel on the blotting paper, wet it with little extra buffer.

v). Add the membrane and remove all bubbles, wet it with little extra buffer.

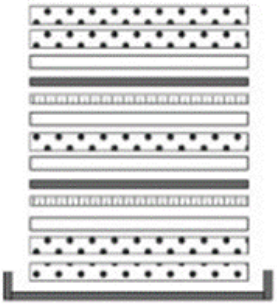
vi). Add two layers of well soaked thick blotting paper (prevent bubbles).

vii). Add two wet sponges (prevent bubbles).

viii). Repeat to add more electroblotting sandwiches.

ix). Close the Western apparatus pressing together the sponges.

x). Follow manufacturer recommendations.



4. Electro-blotting conditions

We recommend using constant current.

Optimized conditions for 6 to 12% gels

*Note that these conditions are optimized for a 1 mm gel and might vary based on your gel composition and thickness. When adapting ReadyBlot™ to your needs, use the prestained markers of your run to observe if there are still protein bands in the gel and correct your transfer conditions accordingly.

Start adjustments by increasing or reducing mA, not time.

Current	Minimal transfer time*
330 mA	25-30 min
400 mA	15-20 min

Storage

Room Temperature. Up to 5 years from date of manufacture.