*Kwik*Blot Buffer

(Cat.# R2001)

Instrument

Shaker: An orbital shaker or a rocker, setting at 50-80 rpm. It's essential that the membrane moves freely inside the tray while shaking in every step.

Buffer Preparation

Decant the full bottle in a large container (such as Cat#. D2002), add 10L water to fully dissolve it. The buffer is good for 3 months if stored at room temperature.

Procedures

(All steps use at least 0.5 mL/cm^2 membrane, and are performed at room temperature.)

Pre-treatment

1. Highly recommended: submerge the membrane in 70% ethanol for 5 minutes. The ethanol solution further exposes the hydrophobic surface of the proteins, making them tightly bound to the hydrophobic membrane. PVDF membrane is more hydrophobic than nitrocellulose membrane. It is a better choice for western blotting.
2. Rinse the membrane with R2001 for 5 minutes to completely wash away the ethanol.

Primary Ab

3. Submerge the membrane in a sufficient volume of R2001. We recommend using 15-20mL for a full-sized mini-blot. A separate blocking step is not needed.
4. Dilute your primary Ab stock in R2001 to make a new stock at 10 µg/mL (see Note 1), and store it at -20°C. Add the primary Ab in Step 3 to 10 ng/mL (1:1,000 dilution). This concentration should work for most antibodies. The optimal working concentration may be obtained by further adjustment.
5. Incubate the membrane on the shaker for as long as your experiment permits. The longer the incubation time, the higher detection sensitivity (see Note 2).
6. Recover the primary Ab solution and store it at 4°C for future reuse. The Ab solution can also be stored at -20°C for long term storage. Typically the antibody solution can

be re-used for more than 5 times. Once the signal starts to go down, spike the solution with the same antibody to make up for the loss of antibody. To reuse the primary Ab solution, simply submerge the membrane in it after Step 2.

Secondary Ab

7. Rinse the blot twice using R2001, each for 15 seconds. Then wash the membrane once on the shaker for 30 minutes. Discard the wash buffer.
8. Then, submerge the membrane in a sufficient volume of R2001. We recommend using 15-20mL for a full-sized mini-blot.
9. Add ***Kwik*Blot** secondary Ab (R2005-2009, R2011-2012) in Step 8 at 1:1,000 ratio. (<u>If the user wish to use own secondary Ab, the user must adjust the antibody dilution factor rather than their routine usage.)</u>
10. Incubate the membrane on the shaker for at least 1 hour, or as long as it permits. The longer the incubation time, the higher detection sensitivity. The background does not increase with longer incubation time.
11. Rinse the blot twice using R2001, each for 15 seconds. Then wash the membrane twice on the shaker for 30 minutes each.

Imaging

12. Rinse the blot with de-ionized water for 15 seconds to remove residual R2001, and transfer the blot onto the sample tray of ***Kwik*Quant** Pro Imager (D1010).
13. Apply 1:5 H_2O-diluted ***Kwik*Quant** Digital-ECL (R1002) mixture (A:B=1:1) on the membrane and wait for 2 minutes.
14. Drain the surface ECL solution, and immediately put the membrane in ***Kwik*Quant** Pro Imager for image acquisition.
15. If the bands are too weak at 2 min exposure, re-apply the non-diluted ***Kwik*Quant** Digital-ECL mixture (A:B=1:1) on the membrane for another 2 minutes, followed by image acquisition.

Final

16. After the image acquisition, the membrane can be stored temporarily in deionized water at 4°C. The image acquisition can be repeated within 24 hours without significant loss of signals. The membrane can also be frozen at -20°C in water for a longer period.
17. The membrane can be renewed for more rounds of probing using ***Kwik*Renew** buffer (Cat.# R2023).

Note 1: The primary Ab's working concentration in R2001 is typically lower than that in milk or BSA solution.

Note 2: The reaction rate of liquid-solid antibody-antigen binding in western blotting is very slow. The time to reach completion is long. In the case provided below, the reaction reached completion at 24 hours (even at room temperature). Increasing the Ab incubation time is the easiest way to achieve higher detection sensitivity.