Cat.#R1100 *Clean*Western Detection Kit
(Membrane blocking step is not required)

Kit Components:

1. Digital Anti-mouse IgG-HRP (1mL)	cat.#R1005
2. Digital Anti-Rabbit IgG-HRP (1mL)	cat.#R1006
3. *Kwik*Quant Ultra HRP Substrate Solution (100mL)	cat.#R1002
4. *Clean*Western Buffer (3.5L)	cat.#R2001

Stability at 4°C: 24 months

Procedures

1. Dilute your primary antibody in Stamina Primary Antibody Dilution Buffer (R2004), and directly add to the membrane (membrane blocking is not necessary). Incubate with shaking 1-24 hours at room temperature. If not using R2004, use 0.5-1% skim milk, although the blot signal may be reduced.

2. Rinse the membrane with dI water twice.

3. Wash the membrane using *Clean*Western Buffer 30 minutes once.

4. Dilute (@1:1000) the Digital HRP-secondary antibody in *Clean*Western Buffer directly (or 0.5-1% skim milk in standard TBST). Probe the membrane with rocking at room temperature for 1-12 hour. The signal is proportionally stronger with longer incubation time.

5. Rinse the membrane with dI water twice.

6. Wash the membrane using *Clean*Western Buffer for 30 minutes once.

7. Rinse the blot with de-ionized water* briefly to remove the residual *Clean*Western Buffer, and transfer the blot onto the sample plate of *Kwik*Quant Imager. (*The buffer contains reagents that will inhibit the reaction of HRP-ECL substrate.)

8. Submerge the membrane in 1:5 H_2O-diluted *Kwik*Quant Digital-ECL mixture (A:B=1:1) for 2 minutes.

9. Put the membrane on a *Kwik*Quant Imager (or any chemiluminescence imagers) for image acquisition.

10. If the bands are too weak at 2 min exposure, submerge the membrane in non-diluted *Kwik*Quant Digital-ECL mixture (A:B=1:1) for another 2 minutes, followed by image acquisition.