

## Western Blot Signal Enhancer Reagent va-100

Immunoblotting (western blot) is widely used to identify specific proteins recognized by polyclonal or monoclonal antibodies. Solubilized protein samples are separated by polyacrylamide gel, and then electrophoretically transferred to a membrane, such as a nitrocellulose. The transferred proteins are bound to the surface of the membrane, providing access for reaction with immunodetection reagents.

To detect low level expressed proteins successfully, it is crucial to increase the sensitivity of detection. Western Blot Signal Enhancer reagent is designed to meet this requirement. Signal Enhancer strongly promote antigen and antibody interactions and offers ultra high signal-to-noise ratios, resulting in outstanding sensitivity and low backgrounds.

### Protocol

Follow the standard procedure to separate your proteins in polyacrylamide gels and transfer them to nitrocellulose membranes.

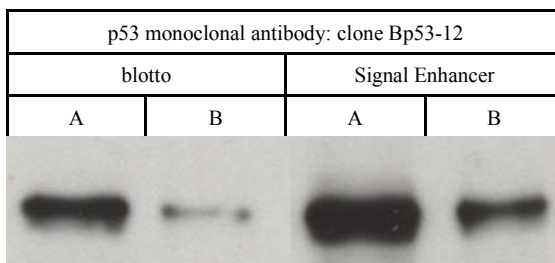
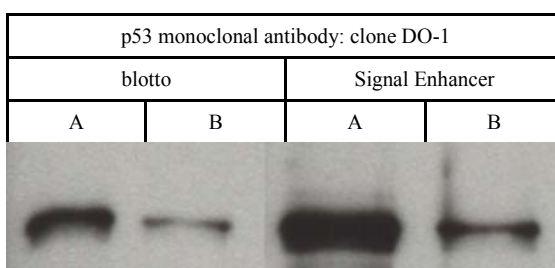
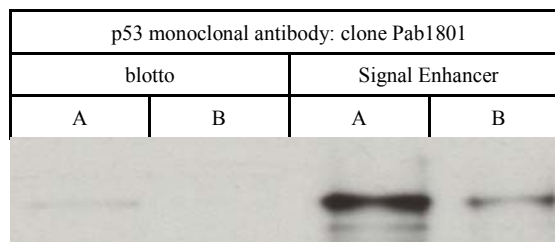
1. Briefly wash the membranes with TBST
2. Block the membranes with 5% Blocking Reagent (va-99) in TBST for 60 min at room temp and gently agitate.
3. Incubate the membranes with primary antibodies diluted with 5% Blocking Reagent (va-99) in Signal Enhancer for 60 min at room temp and gently agitate.
4. Wash the membranes 3 x 5 min with TBST.
5. Incubate the membranes with secondary antibodies diluted with 5% Blocking Reagent (va-99) in Signal Enhancer for 60 min at room temp and gently agitate.
6. Wash the membranes 4 x 5 min with TBST.
7. Briefly wash the membranes with TBS.
8. Visualize signals with Chemiluminescence Luminol, or other standard protocols. If luminol is used for visualization, HRP-conjugated secondary antibody must be used.

Note: 5% Blocking Reagent be prepared fresh on the day of use.

### Research Use

For research use only, not for use in diagnostic procedures.

### Data



### Comparisons of Signal Enhancer with standard blotto by western blotting

4ug (Lane **A**) or 1ug (Lane **B**) of A431 whole cell lysis is loaded in SDS-PAGE gels, then following the standard procedure to separate and transfer the proteins on to nitrocellulose membranes. After blocking with 5% non-fat milk in TBST (blotto), the membranes are probed with monoclonal antibodies against p53 at 0.2ug/ml (Panel 1 and 2 with Pab1801; Panel 3 and 4 with DO-1; Panel 5 and 6 with Bp53-12). The membranes are then incubated with HRP conjugated goat anti-mouse antibodies. Both of primary and secondary antibodies are diluted with either blotto (blotto panels) or Signal Enhancer (Signal Enhancer panels).

### Product

Each bottle contains 250 ml of the reagent. Store at 4° C and stable for 12 months from the date of shipment.

### Related Products

Blocking Reagent: va-99  
Luminol Reagent: va-98