Western Blot Enhancing Kit (Cat. W212010)

Product Information:

Contents: Affinity enhancer buffers (Cat: A212060, 100mL); Western blot blocking buffer (Cat: B208060, 20g);

Catalog Number: W212010 *Use for research only*

Size: 10 assays Stored at 2-8°C

Description: Design for effectively blocking the non-specific sites and enhancing the antigen-antibody specific bindings during western blotting, ELISA and IPs, suitable for both PVDF and NC membranes. Specific applications on some poorly reactive or low-reactive primary antibodies (Monoclonal), which may cause super-low signals, faint bands or high non-specific noises.

Procedure: (prepare membrane after protein wet transfer)

1. Preparation of blocking buffer for membrane blocking and antibody dilution
   - Shake the blockers for several times to ensure even distribution of components.
   - Dissolve 2g blockers in 100ml of 1x PBST/TBST, pH7.5. Shake or stir to fully dissolve.

   Note*: This blocking buffer is ready to be used for blocking or antibody diluent and may be stored at 2-8 ºC, but should be used within 24 hours.
   The blocking buffer contains biotin and should not be used for the avidin-biotin systems.
2. Preparation of the affinity enhancing buffer
   - Mix equal volumes of affinity enhancer buffer A and B in a plastic container.

   Note*: The mixture of 5-7.5ml of buffer A and 5-7.5ml of buffer B is enough for a mini-gel membrane (8x10cm).
3. Blocking: (membrane after protein wet transfer)
   - Wash membrane with 1x TBST/PBST twice for 5 minutes with gentle shaking.
   - Submerge membrane in blocking buffer and incubate for 40 minutes at room temperature with gentle shaking.
4. The primary (1st) antibody affinity enhancing procedure
   - Wash membrane with 1x TBST/PBST twice for 5 minutes with gentle shaking.
   - Incubate membrane in the primary (1st) antibody (diluted with blocking buffer) for 40 minutes at room temperature or overnight at 2-8°C with gentle shaking.
   - Wash membrane with 1x TBST/PBST twice for 5 minutes.
   - Immerse membrane in the plastic container contained the mixture of the affinity enhancing buffer. Incubate at room temperature for 10 minutes with gentle shaking.
   - Wash membrane with 1x PBST/TBST twice for 5 minutes.
   - Incubate membrane in the primary (1st) antibody (diluted with blocking buffer) for 40-90 minutes at room temperature with gentle shaking.
5. Wash membrane twice with 1x PBST/TBST for 5 minutes.
6. Incubate membrane in the secondary (2nd) antibody (diluted with blocking buffer) for 1 hour at room temperature with gentle shaking.
7. Wash membrane twice with 1x PBST/TBST for 5 minutes and detected by chemiluminescence substrates (ECL).

Precautions and Disclaimer: This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.

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