DATA SHEET

Instructions for ImmunoBooster™ IFA, IHC, IP

Important Product Information

- The stability of primary antibodies diluted in the ImmunoBooster ELISA Antibody Diluent varies. For best results, dilute the antibody in the working solution no more than 1 hour before use.
- It is recommended as good laboratory practice to use gloves and personal protective gears, especially as serum samples used in ELISA assays may be infectious.

Material Included

- **ImmunoBooster for IFA, IHC, IP (**ready solution**). Catalog #: BIFA-50**

Additional Materials Required

- Primary Antibody: Choose a mouse, rabbit or human antibody that is specific to the target protein(s). The optimal dilution to use depends on the specific primary antibody. However, in our experience the same dilution which was used with the traditional assay works well. In some cases, the primary antibody can be diluted 2 to 4-fold more with comparable results to the traditional assay.

Procedure for IHC and IFA

1. Dilute your primary antibody in ImmunoBooster according to dilutions used in regular IHC or IFA.
2. Add ~50 to100 µl of diluted antibody to the slide to cover the sample and incubate for 10 minutes at RT (incubation time can be extended to no more than 15 minutes)
3. Wash the slides four times with PBS or TBS (or use your regular wash method).
4. Immediately add 50-100 µl of Secondary Antibody diluted in the ImmunoBooster to cover the sample and incubate for 10 minutes at RT.
5. Wash the wells four times with PBS or TBS (or use your regular wash method).
6. Follow up with regular staining and counter staining method according to your IHC or IFA protocol.

Procedure for IP

1. Mix 10-500 µg cell lysate plus the recommended amount of antibody, then add four volumes of ImmunoBooster and vortex for 5 seconds.
2. Resuspend the recommended amount of washed Protein-A or Protein-G coupled beads in 100µl of ImmunoBooster then add to the tube with your antigen antibody mixture.
3. Incubate under rotatory agitation at room temperature for 15 minutes.
4. When the incubation time is over, centrifuge the tubes, remove the supernatant and wash the beads with lysis buffer three times (each time centrifuging and removing the supernatant).Wash the wells four times with PBS or TBS.
5. Finally, remove the last supernatant and add 25-50 µl of 2x loading buffer. Boil at 95-100°C for 5 minutes to denature the protein and separate it from the protein-A/G beads, then centrifuge and keep the supernatant where the protein is now. You can then freeze the samples or run them on a SDS-PAGE.

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