Bridging Antibody for Mouse IgG

Catalog No.: 53017
Format: 500 µg
Storage: Store at -20°C. Guaranteed stable for 6 months from arrival when stored properly.

Description: Certain isotypes of mouse antibody do not bind efficiently to protein G-conjugated agarose or magnetic beads. The Bridging Antibody is a designed to improve the binding of protein G beads to mouse antibodies, thus improving the yield of chromatin immunoprecipitation and protein immunoprecipitation experiments.

Quality Control: The Bridging Antibody has been tested for effectiveness in improving results of chromatin immunoprecipitation experiments using mouse IgG primary antibodies.

Bridging Protocol

Step 1: Incubate beads with the bridging antibody

1. Pipet the appropriate amount of beads (25 µl for each ChIP application if using Active Motif's ChIP-IT™ Express or Protein G Magnetic Beads) in a 1.7 ml microcentrifuge tube and add 5 µl (5 µg) of Bridging Antibody per IP reaction. Mix well by pipetting up and down, then cap the tubes.
2. Incubate for 1 hour at 4°C on a rolling shaker or rotating mixer.

Step 2: Wash protein G beads

1. Briefly spin tubes in a microcentrifuge to collect liquid from caps.
2. Place tubes in the magnetic stand and allow beads to pellet on tube side. (Or, if using protein G-conjugated agarose beads, pellet the beads for 1 minute in a microcentrifuge at 1,000 x *g*).
3. Carefully remove the supernatant and discard.
4. Add 200 µl of PBS per IP (or ChIP Buffer 1) and completely resuspend pellet by pipetting up and down several times.

   Note: Take care to ensure that beads are not clinging to the pipet tips after pipetting. It may be necessary to move the tubes away from the magnetic field before resuspending.
5. Place tubes in the magnetic stand and allow beads to pellet on tube side. (Or, if using protein G-conjugated agarose beads, pellet the beads for 1 minute in a microcentrifuge at 1,000 x *g*).
6. Carefully remove the supernatant and discard.
7. Resuspend beads in the appropriate amount of PBS or ChIP Buffer 1 (25 µl for each ChIP application if using ChIP-IT Express or Active Motif's Protein G Magnetic Beads).

The beads/bridging antibody complex can now be used in IP, ChIP or other applications that use mouse IgG primary antibodies.

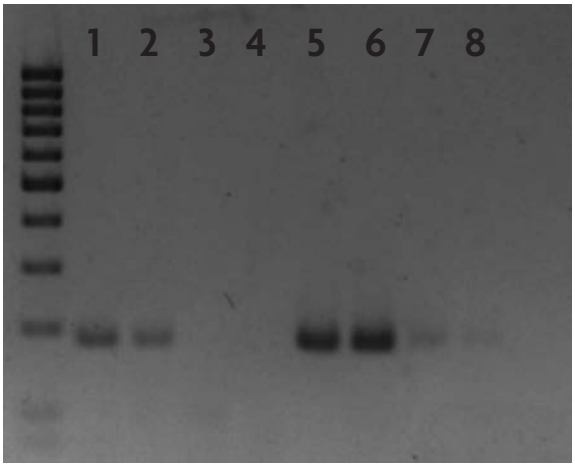

Figure 1: Improvement in Chromatin IP using anti-mouse Bridging Antibody. ChIP was performed using chromatin from U-937 cells induced with TNF-α (10 ng/ml for one hour). PCR was performed with primers corresponding to the human IL-8 promoter.

Lanes 1-4: Beads pre-incubated with no bridging antibody.
Lanes 5-8: Beads pre-incubated with 5 µg bridging antibody.
Lanes 1, 2, 5 & 6: ChIP performed using p65 mouse monoclonal antibody, 2 µg per IP.
Lanes 3, 4, 7 & 8: ChIP performed using negative control mouse IgG.