Crosslinking

The protocol presented below is an example using one of several commercially available cross-linkers.

1. Add 1 ml 0.2 M triethanolamine, pH 8.2 to the Dynabeads - Ig complex with immobilised immunoglobulin. Wash twice according to Washing Procedure below, using 0.2 M triethanolamine, pH 8.2 as the washing buffer.
2. Resuspend the Dynabeads - Ig complex in 1 ml of 20 mM DMP (dimetyl pimelimidate dihydrochloride, Pierce #21666) in 0.2 M triethanolamine, pH 8.2 (5.4 mg DMP/ml buffer). This crosslinking solution must be prepared immediately before adding to the Dynabeads - Ig complex.
3. Incubate with rotational mixing for 30 minutes at 20° C. Place the tube on the magnet and discard the supernatant.
4. Remove the tube from the magnet and stop the reaction by resuspending the Dynabeads - Ig complex in 1 ml of 50 mM Tris, pH 7.5 and incubate for 15 minutes with rotational mixing.
5. Place the tube on the magnet and discard the supernatant.
6. Wash the now crosslinked Dynabeads - Ig complex 3 times with 1 ml PBS pH 7.4 by the use of a magnet, according to Washing Procedure below. Resuspend the Dynabeads - Ig complex to 100 µl or add directly to antigen-containing solution. The full recovery of your Ig activity cannot be guaranteed, as this varies from Ig to Ig.

NOTE: The protocol presented here uses 0.2 M triethanolamine pH 8.2. Other non-amine containing buffers with pH 7-9 can also be used.

Washing Procedure

The washing procedure is facilitated by the use of a magnet (DynaMag-2).

1. Resuspend the Dynabeads Protein A, thoroughly in the vial (e.g. by vortexing 1-2 minutes or rotating on a roller) to obtain a homogeneous suspension.
2. Transfer 100 µl Dynabeads Protein A to a test tube at room temperature. (Please refer to section 1.3 above for details on binding capacity.)
3. Place the test tube on the magnet for one minute and pipette off the supernatant.
4. Remove the test tube from the magnet and add 0.5 ml 0.1 M Na-phosphate buffer pH 8. 5. Repeat steps 3, 4 and 3.