Steinbrenner Laborsysteme GmbH

magtivio

Technical Note

IgG crosslinking to MagSi-protein A and MagSi-protein G magnetic beads

I. Introduction

This protocol deals with the covalent cross-linking of the IgG to the Protein A and MagSi-protein G coated magnetic beads.

II. Materials/solutions

1. 0.01M Phosphate buffered saline with Tween20 (PBS-Tween 20).
2. PBS-Tween 20 with 150 mM NaCl.
3. MagSi-protein A or MagSi-protein G magnetic beads.
4. Elution buffer: 0.1 M glycine-HCl (pH 2.6).
5. Crosslinking buffer: 0.2 M triethanolamine in PBS (pH 8-9).
6. DMP crosslinking solution: Dimethyl pimelidate dihydrochloride/Cross-linker (DMP) (Sigma, D-8388) (10 mg DMP in 2ml crosslinking buffer).
7. DMP Blocking buffer: 0.1 M ethanolamine in PBS (pH 8-9).
8. Neutralization buffer: 1M TRIS pH 9 (pH set with HCl 37%).
9. IgG (immunoglobulins).
10. Vortex mixer, rotator, microcentrifuge tubes, magnetic separator, pipettes and tips.

III. Protocol

A) IgG binding

1. Vortex and resuspend MagSi-protein A or G beads.
2. Aliquot 100 µl of MagSi-protein A or G to a microcentrifuge tube (2 ml).
3. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

4.

5. Repeat step 2-3 two times for a total of 3 washes. Resuspend the beads in 90 µl PBS-Tween 20 with 150mM NaCl.

6. Add 20 µg IgG in a maximum volume of 50 µl solution (pH 8-9) and mix by vortexing. Incubate for 30 minutes at RT an mix by vortexing every 5 minutes.

7. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

8. Add 300 µl PBS-Tween20 with 150 mM NaCl and mix by vortexing.

9. Repeat step 6-7 once more with PBS and once with ddH2O for a total of 3 washes.

B) Crosslinking

1. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

2. Pre-rinse the beads with 300µl 0.2M triethanolamine pH 8.2 and mix by vortexing.

3. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

4. Add 300 µl DMP crosslinking solution and mix by vortexing. Incubate for 30 min at RT and mix by vortexing every 5 minutes.

5. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

6. Repeat steps 4-5 one more time to increase crosslinking efficiency.

7. Add 300 µl DMP blocking buffer and mix by vortexing.

8. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

9. Add 300 µl DMP blocking buffer and mix by vortexing. Incubate 1h under rotation. Mix every 10 minutes by vortexing.

10. Place the tube on the MM-Separator M12+12, collect the beads for 3 minutes and discard the supernatant.

11. Add 300 µl PBS and mix by vortexing.

12. Repeat steps 10-11 once more with PBS and once with ddH2O.

13. Finally store the beads in 100 µl PBS-Tween 20, 0.1% sodium azide.