micro sphere technology

Method for Coupling IgG to Microsphere Technology FERROSPHERE-N

Activation:

1) Weigh out 5mg of dry amine-functionalised FERROSPHERES-N (MTL/FER) into a 1.5mL microcentrifuge tube.

2) Wash 6 times (1mL/wash) with 10mM phosphate buffered saline, pH 7.2, 150mM sodium chloride (PBS), using a Dynal MPC™-S magnetic separator, or similar. Remove and replace the liquid carefully each time using a 1mL pipette.

3) Add 1mL of 10% glutaraldehyde in PBS (we recommend the use of Polysciences, Inc. 50% glutaraldehyde (Cat #: 18428), because of its high purity/low polymer content).

4) Incubate at room temperature for 2 hours with constant agitation (we recommend using a rotating blood tube wheel (Stuart rotator SB3, or similar) set to 30 rpm to ensure good, end over end, mixing).

5) Wash the FERROSPHERES-N 6 times with PBS as above.

Antibody Coupling:

6) FERROSPHERES-N have an IgG coupling capacity of 2-4mg/g. We recommend that IgG be added to the FERROSPHERES-N at a 5 to 10 fold excess of this coupling. Add the IgG to the FERROSPHERES-N in a total volume of 1mL, the extra volume being made up with PBS.

7) Incubate at room temperature for 18 hours with constant agitation on the tube rotator (for this extended incubation period, we recommend a setting of 15rpm).

8) Use the magnetic separator to pull the FERROSPHERES-N to the side of the tube and add 25μL of 1M sodium cyanoborohydride (dissolved in deionised water) to the FERROSPHERES-N/PBS mixture. Cyanoborohydride is a reducing agent and as such may impact on the activity of some antibodies. It is advisable to assess this beforehand.

9) Remove the tube from the magnetic separator and incubate at room temperature with constant agitation on the tube rotator (15rpm) for a further 30min.

10) Use the magnetic separator to pull the FERROSPHERES-N to the side of the tube and add 45μL of 100mg/mL adipic dihydrazide (adipic acid hydrazide), dissolved in deionised water, to the FERROSPHERES-N/PBS/cyanoborohydride mixture. The dihydrazide may require a little warming to dissolve in water at this concentration.

11) Remove the tube from the magnetic separator and incubate at room temperature with constant agitation on the tube rotator (15rpm) for a further 30min.

12) Wash the FERROSPHERES-N 6 times with PBS as above.

Removal of non-specifically bound antibody:

13) Wash the FERROSPHERES-N 3 times with deionised water.

14) Wash the FERROSPHERES-N 1 time with 50mM sodium acetate, pH 4.0, containing 300mM sodium chloride.

15) Wash the FERROSPHERES-N 1 time with deionised water.

16) Wash the FERROSPHERES-N 1 time with 50mM sodium carbonate, pH 9.0, containing 300mM sodium chloride.

17) Wash the FERROSPHERES-N 1 time with deionised water.

18) Repeat steps 14-17 a further 2 times.

19) Wash the FERROSPHERES-N with the storage buffer of choice and store in this buffer at 4 degrees Celsius.

Note: Capacity measurements were carried out by assaying the IgG coupled FERROSPHERES-N after the final water wash, using the Pierce BCA assay

The procedure described can be scaled up as required, but would require the use of a Dynal MPC™-1 magnetic separator (or similar).

Microsphere Technology Ltd., Adare, Co. Limerick, Ireland
tel: + 44 (0) 131 440 1884 fax: + 44 (0) 131 448 1111
www.microspheretechnology.com info@microspheretechnology.com