SPHERO™ Technical Note **STN-11 Rev B 081607**

Magnetic Particle Coated with Pepsin and Papain

Preparation of IgG F(ab')2 Fraction Using Pepsin Coated Magnetic Particles (Spherotech Cat. # PEPM-40-2)

The Spherotech Cat. No. PEPM-40 consists of 4µm magnetic particles covalently linked to pepsin from porcine stomach mucosa. These particles efficiently cleave IgG from various species producing F(ab')2 and other lower molecular weight products within a few hours. After digestion Pepsin-Magnetic particles can be easily removed magnetically from the reaction vessel leaving no pepsin in the solution. As a result, the cleavage of IgG can be effectively controlled. The supernatant containing products from the cleavage and any residual IgG can be further purified chromatographically or by using Protein A magnetic particles (Spherotech Cat. # PAMX-10).

This procedure allows the controlled preparation of essentially pure F(ab')2 in simple two steps. In addition, pepsin magnetic particles can be reused repeatedly without a significant loss in activity.

Protocol:
Materials Needed:
1. 0.01M acetate buffer, pH 3.0
2. Spherotech Flexi-Mag Magnetic Separator (Spherotech Cat. # FMJ-1000)
3. Incubator set at 37 °C.
4. Rotator or stirrer.
5. Protein A coated magnetic particles (Spherotech Cat # PAMX-10)

Procedure:
1. Make an IgG solution in 0.01M acetate buffer, pH 3.0. The preferred concentration is 1mg/mL.
2. Transfer 200-500µL of Pepsin-magnetic particles (1% w/v) for each mg of IgG to a screw capped vial or tube.
3. Separate the particles magnetically. Remove the supernatant. Wash the particles 1 time with acetate buffer, pH 3.0.
4. Add IgG solution in acetate buffer, pH 3.0 to the washed Pepsin-magnetic particle pellet.
5. Cap, mix and rotate or stir gently for 2 to 24 hours at room temperature or 37°C. The duration of incubation can be adjusted to achieve the desired level of IgG cleavage.
6. Magnetically separate the particles and collect the supernatant. Transfer the particles to a vessel containing the neutralizing solution (e.g. ~ 30µL of 10N NaOH for each mL of solution).
7. Add Protein A magnetic particles (PAMX-10) to the supernatant from step 6 to bind and remove residual IgG and Fc fractions from the solution.
8. The supernatant remaining after PAMX-10 treatment will contain mostly F(ab')2 fragments.

Note:
- Each mL of PAMX-10 will bind to 30-50µg of human, mouse and rabbit IgG; however, IgG of some species such as rat and goat will not bind to protein A.
- Collect the Pepsin magnetic particles and Protein A magnetic particles (PAMX-10) for reuse. Wash particle pellet with PBS or other neutral buffer and store refrigerated.

Preparation of IgG Fab Fraction Using Papain Coated Magnetic Particles (Spherotech Cat. # PAPM-40-2)

The Spherotech PAPM-40 consists of magnetic particles (4µm) covalently linked to Papain from Papaya latex. These particles efficiently cleave IgG into Fab and Fc fragments within a few hours. After digestion Papain-Magnetic particles can be easily removed from the reaction vessel magnetically leaving no Papain in the solution. As a result, effectively stopping further cleavage of IgG. The supernatant containing products of cleavage and any undigested IgG can be further purified chromatographically.

This procedure allows the controlled preparation of essentially pure Fab in simple two steps. In addition, papain magnetic particles can be reused repeatedly without a significant loss in activity.

Protocol:

Materials Needed:

1. 0.01M acetate buffer, pH 5.0
2. 20mM NaEDTA solution in water
3. 1M L-Cysteine solution in 1N HCl
4. 0.5M Iodoacetamide solution in water
5. Spherotech Flexi-Mag Magnetic Separator (Spherotech Cat. # FMJ-1000)
6. Incubator set at 37°C.
7. Rotator or stirrer.

Procedure:

1. Make an IgG solution in 0.01M acetate buffer, pH 5.0. Add 50uL of EDTA and 25µL of L-Cysteine solutions to each mL of IgG solution. Preferred concentration of IgG is 1mg/mL.
2. Transfer 200-500µL of Papain-magnetic particles (1% w/v) for each mg of IgG to a screw capped vial or tube.
3. Separate the particles magnetically. Remove the supernatant. Wash the particles 1 time with acetate buffer, pH 5.0.
4. Add the IgG solution in acetate buffer, pH 5.0 to the washed Pepsin-magnetic particle pellet.
5. Cap and mix by rotation or stirring gently for 2 to 24 hours at room temperature or 37°C. The duration of incubation can be adjusted to achieve the desired level of IgG cleavage.
6. Magnetically separate the particles and collect the supernatant. Add 100 µL of Iodoacetamide solution to each mL of supernatant to stabilize the Fab.

Note: Collect the Papain-magnetic particles for reuse. Wash the particle pellet with PBS or other neutral buffer. Refrigerated the particles during storage.

Why Magnetic Particle Coated Enzymes ?

Several advantages of using enzymes covalently linked to magnetic particles over enzymes attached to non magnetic gel, latex, or soluble enzymes are listed below:

1. The magnetic separation of enzymes covalently linked to magnetic particles stops the cleavage of IgG without additional inhibitors, pH changes or other extraneous agents. This avoids contamination of the sample.
2. Magnetic particles coated with enzymes are can be reused repeatedly without any significant loss in enzymatic activity.
3. The reaction kinetics of small uniform magnetic particles coated with various enzymes is fast and efficient.

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