

Magnetic Particles Coated with Pepsin, Papain and Trypsin

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

PEPM-40 consists of 4 μ 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; effectively stopping further cleavage of IgG. Supernatant containing products of cleavage, and any residual IgG, may be further purified chromatographically or with the help of Protein A magnetic particles (Cat. # PAMX-10). This procedure allows preparation of essentially pure F(ab')₂ in simple two steps in a controlled fashion. Pepsin Magnetic Particles can be reused repeatedly without a significant loss in activity.

Protocol:

Materials Needed:

1. 0.01 M acetate buffer pH 3.0
2. Magnetic separator (Cat. # FMJ-1000)
3. Incubator set to 37 °C.
4. Rotator or stirrer.
5. Protein A coated magnetic particles (Cat # PAMX-10)

Procedure:

1. Make IgG solution in 0.01 M acetate buffer pH 3.0. Preferred concentration is 1 mg/mL.
2. For each mg of IgG 200-500 μ L of Pepsin-magnetic particles (1% w/v) are needed.
3. Transfer calculated amounts of particles to a screw capped vial or tube, separate particles magnetically. Remove supernatant. Wash particles 1X with pH 3.0 acetate buffer.
4. Add IgG solution in acetate buffer pH 3.0 from step 1 to washed Pepsin-magnetic particle pellet in step 3.
5. Cap, mix and rotate or stir gently for 2 to 24 hours at 37°C. Duration of incubation and temperature (room temperature or 37°C) may be adjusted to give desired level of IgG cleavage.
6. Magnetically separate particles and collect supernatant and transfer it to a vessel containing neutralizing solution (e.g. ~ 30 μ L of 10 N-NaOH for each mL of solution).
7. To the supernatant from step 6 add Protein A magnetic particles (PAMX-10) to bind and remove residual IgG and Fc fractions from the solution.

Note:

- Each mL of PAMX-10 will bind to 30-50 μ g of human, mouse and rabbit IgG, however IgG of some species such as rat and goat will not bind to protein A.
 - Save Pepsin-magnetic particles and Protein A-magnetic particles (PAMX-10) for reuse. Wash particle pellet with PBS or other neutral buffer and store refrigerated.
8. Supernatant remaining after PAMX-10 treatment will contain mostly F(ab')₂ fragments.

SpheroTECHNICAL NOTES

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Preparation of IgG Fab Fraction Using Papain Coated Magnetic Particles

(Cat. # PAPM-40-2)

PAPM-40 consists of magnetic particles (4 μ) covalently linked to Papain from Papaya latex used to 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, effectively stopping further cleavage of IgG. Supernatant containing products of cleavage, and any residual undigested IgG, may be further purified chromatographically. This procedure allows preparation of essentially pure Fab in simple two steps in a controlled fashion. Papain Magnetic Particles can be reused repeatedly without a significant loss in activity.

Protocol:

Materials Needed:

1. 0.01 M acetate buffer pH 5.0
2. 20 mM EDTA Na solution in water
3. 1 M L- Cysteine solution in 1 N HCl
4. 0.5 M Iodoacetamide solution in water
5. Magnetic separator (Cat. # FMJ-1000)
6. Incubator set to 37 °C.
7. Rotator or stirrer.

Procedure:

1. Make IgG solution in 0.01 M acetate buffer pH 5.0. Add 50 μ L of EDTA and 25 μ L of L-Cysteine solutions to each mL of IgG solution. Preferred concentration of IgG is 1 mg/mL.
2. For each mg of IgG 200-500 μ L of Papain -magnetic particles (1% w/v) are needed.
3. Transfer calculated amounts of particles to a screw capped vial or tube and separate particles magnetically. Remove supernatant. Wash particles once with pH 5.0 acetate buffer.
4. Add IgG solution from step 1 to Pepsin-magnetic particle pellet in step 3.
5. Cap and mix by rotation or stirring gently for 2 to 24 hours at room temperature or 37°C. Duration of incubation may be adjusted to give desired level of IgG cleavage.
6. Magnetically separate particles and collect supernatant. To stabilize Fab add 100 μ L of Iodoacetamide solution to each mL of supernatant.

Note: Save Papain-magnetic particles for repeat use. Wash particle pellet with PBS or other neutral buffer and store refrigerated.

Proteolysis Using Using Trypsin Coated Magnetic Particles

(Cat. # TRPM-40-2)

TRPM-40 magnetic particles (4 μ) are covalently linked to Trypsin from bovine pancreas and are useful as general purpose proteolysis at neutral pH. TRPM-40 pellet of 200 μ L particle suspension caused a >90% reduction in OD 630 of a 2.5% solution of non fat milk solution in 1 hour. Among many applications TRPM-40 may be used to digest formalin fixed tissue before nucleic acid amplification similarly it may be useful in reducing or eliminating proteins from sugar or other solutions where proteins or enzymes are not desired without leaving enzyme in solution. There are many potential applications but any specific use will depend on the specific purpose.

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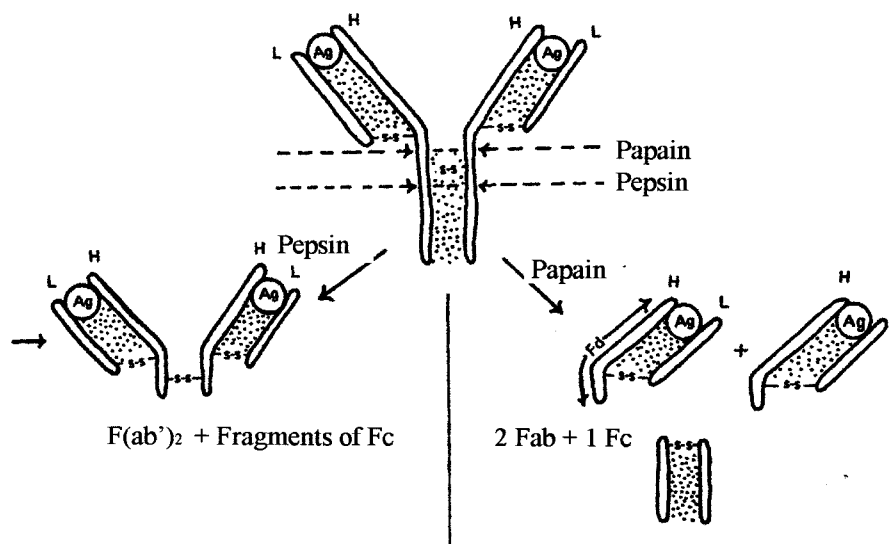
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Why Magnetic Particle Coated Enzymes ?

There are several advantages of using Enzymes covalently linked to magnetic Particles over enzymes attached to non magnetic gel or latex or soluble forms of enzymes; some are listed below:

1. Nearly complete removal of enzyme after the reaction allows reaction to stop immediately without addition of inhibitors, changing pH or other extraneous agents to reaction mixture. This avoids contamination of the sample.
2. Magnetic particles coated with enzymes are economical because they can be reused repeatedly without any significant loss in enzymatic activity.
3. Reaction kinetics of small uniform magnetic particles coated with various enzymes is fast and efficient.

Schematic of IgG Cleavage by Pepsin and Papain Magnetic Particles



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