Spleen Dissociation Medium

Medium for dissociation of mouse spleen

Catalog # 07915 10 x 4 mL

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Product Description

This product is designed to maximize the recovery of dendritic cells from mouse spleen when combined with EasySep™ cell separation technology. This medium contains collagenase, DNase, and fetal bovine serum (FBS), and has been optimized for maximum viability of isolated spleen dendritic cells. Each 4 mL tube is sufficient for processing up to two mouse spleens.

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:
- Collagenase IV
- DNase
- Fetal bovine serum (FBS)
- Roswell Park Memorial Institute (RPMI) medium

Materials Required But Not Included

- 60 mm Treated Tissue Culture Dishes (Catalog #27120)
- Blunt-End Needles, 16 Gauge (Catalog #28110)
- 3 cc Syringes (Catalog #28230)
- 70 µm nylon mesh filter (e.g. Catalog #27216/27260)

Handling / Directions For Use

Please refer to the appropriate EasySep™ Product Information Sheet (PIS) for recommended medium and cell resuspension concentration.

DISSOCIATION AT ROOM TEMPERATURE (15 - 25°C)

1. Thaw individual tubes of Spleen Dissociation Medium at room temperature (15 - 25°C) and use immediately. Do not re-freeze.
2. In a 60 mm Treated Tissue Culture Dish, mince 1 - 2 freshly isolated spleens into a homogeneous paste using dissection scissors and forceps. Spleen fragments should be less than 1 mm in size.
3. Pour the contents of a 4 mL tube of Spleen Dissociation Medium into the dish and mix well. Using a 5 mL serological pipette (e.g. Catalog #38003), return all suspended spleen fragments and Spleen Dissociation Medium to the original tube.
4. Incubate the tube at room temparature for 30 minutes.
   NOTE: For best results, place the tube horizontally on a rocking platform with continuous agitation. Alternatively, gentle agitation every 5 minutes during the incubation is acceptable.
5. If performing downstream DNase treatment (see kit-specific PIS for details), skip this step and continue to step 6. Otherwise, add EDTA to a final concentration of 10 mM (e.g. 80 µL of a 0.5 M stock), mix, and incubate the dish at room temperature for 5 minutes.
6. Dissociate spleen fragments into a smooth suspension by gently passing several times through a 16 Gauge Blunt-End Needle attached to a 3 cc Syringe.
7. Pour the entire suspension through a primed 70 µm nylon mesh filter into a 50 mL conical tube (e.g. Catalog #38010).
   NOTE: To prime, pass 5 mL of recommended medium through the mesh filter.
8. Rinse the empty Spleen Dissociation Medium tube and mesh filter with an additional 10 mL of recommended medium and add to the 50 mL conical tube.
9. Centrifuge the cell suspension at 300 x g for 10 minutes.
10. Discard supernatant and resuspend cells in appropriate amount of recommended medium. The cells are now ready for downstream applications.

DISSOCIATION AT 37°C

IMPORTANT NOTE: This protocol has been optimized for use with certain EasySep™ cell separation kits. When 37°C spleen digestion is recommended in the kit-specific PIS, follow the protocol below.

1. Thaw individual tubes of Spleen Dissociation Medium at 37°C and use immediately. Do not re-freeze.
2. In a 60 mm Treated Tissue Culture Dish, mince 1 - 2 freshly isolated spleens into a homogeneous paste using dissection scissors and forceps. Spleen fragments should be less than 1 mm in size.
3. Pour the contents of a 4 mL tube of Spleen Dissociation Medium into the dish and mix well.
4. Incubate the dish at 37°C for 30 minutes.
5. If performing downstream DNase treatment (see kit-specific PIS for details), skip this step and continue to step 6. Otherwise, add EDTA to a final concentration of 10 mM (e.g. 80 µL of a 0.5 M stock), mix, and incubate the dish at room temperature (15 - 25°C) for 5 minutes.
6. Dissociate spleen fragments into a smooth suspension by gently passing several times through a 16 Gauge Blunt-End Needle attached to a 3 cc Syringe.
7. Pour the entire suspension through a primed 70 µm nylon mesh filter into a 50 mL conical tube (e.g. Catalog #38010).
   NOTE: To prime, pass 5 mL of recommended medium through the mesh filter.
8. Rinse the empty dish and mesh filter with an additional 10 mL of recommended medium and add to the 50 mL conical tube.
9. Centrifuge the cell suspension at 300 x g for 10 minutes.
10. Discard supernatant and resuspend cells in appropriate amount of recommended medium (see appropriate EasySep™ PIS). The cells are now ready for downstream applications.