

Spleen Dissociation Medium



Medium for Dissociation of Mouse Spleen

Catalog # 07915

10 x 4 mL

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FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.

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 enough to process 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

Handling / Directions For Use

Please see appropriate EasySep™ kit Product Information Sheet (PIS) for recommended medium and cell resuspension concentration.

DISSOCIATION AT ROOM TEMPERATURE (15 - 25°C):

1. Thaw individual tubes at room temperature (15 - 25°C) and use immediately. Do not re-freeze.
2. In a 60 mm Treated Tissue Culture Dish, use dissection scissors and forceps to mince 1 - 2 freshly isolated spleens into a homogeneous paste. 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 pipette, return all suspended spleen fragments and Spleen Dissociation Medium to the original tube.
4. Incubate the tube for 30 minutes at room temperature.
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 screw-cap tube.
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 50 mL conical tube 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, please follow the protocol listed below:

1. Thaw individual tubes at 37°C and use immediately. Do not re-freeze.
2. In a 60 mm Treated Tissue Culture Dish, use dissection scissors and forceps to mince 1 - 2 freshly isolated spleens into a homogeneous paste. 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 for 30 minutes at 37°C.
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 screw-cap tube.
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 50 mL conical tube at 300 x g for 10 minutes.
10. Discard supernatant and resuspend cells in appropriate amount of recommended medium (please see appropriate EasySep™ kit Product Information Sheet for specific instructions). The cells are now ready for downstream applications.

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