

Collagenase Type I (0.25%)

Cell dissociation reagent

Catalog # 07902

5 mL



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Product Description

Collagenase Type I (0.25%) is recommended for tissue dissociation to prepare cell suspensions, dissociation of mouse embryoid bodies (EBs), and solubilization of collagen gels.

Properties

- Storage:** Store at -20°C.
- Shelf Life:** Stable until expiry date (EXP) on label.
- Contains:**
- Collagenase (0.25%)
 - Phosphate-buffered saline (PBS)
 - Fetal bovine serum (FBS) (20%)

Handling / Directions For Use

For complete instructions, refer to the Technical Manual: In Vitro Hematopoietic Differentiation of Mouse ES & iPS Cells Using ES-Cult™ (Document #28415), available at www.stemcell.com or contact us to request a copy.

DISRUPTION OF EBs:

1. Thaw Collagenase Type I (0.25%) at room temperature (15 - 25°C).
NOTE: Once thawed, aliquot into working volumes and store at -20°C. After thawing aliquots, do not re-freeze.
2. Harvest EBs and wash in Iscove's MDM with 2% FBS (Catalog #07700). Remove supernatant carefully as to not disturb the loose pellet.
3. Add 2 - 3 mL of Collagenase Type I (0.25%) and incubate at 37°C for 1 hour.
4. Disrupt EBs by passaging medium and cells through a 21 gauge, 1-inch needle on a 3 cc syringe (3 times).
5. Add Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) with 5% FBS to neutralize the Collagenase Type I (0.25%) and centrifuge at 300 x g for 5 - 8 minutes. Remove and discard supernatant.
6. Resuspend cells in a minimal volume of Iscove's MDM with 2% FBS.

SOLUBILIZATION OF THIN COLLAGEN TYPE I GELS:

Applications include recovery of cells cultured in MegaCult™ collagen-based medium and collagen gels prepared using Collagen Solution (Catalog #04902).

1. Thaw Collagenase Type I (0.25%) at room temperature (15 - 25°C).
2. For a 1 - 1.5 mL collagen thin gel, add 1 mL of Collagenase Type I (0.25%).
3. Incubate at 37°C for 15 - 60 minutes. The optimal time will vary depending on the density of the collagen gel and the cell type being isolated.
NOTE: The appropriate culture medium is dependent on the cell type being isolated and the downstream application. For example, if the isolated cells will be set up in culture, use the same culture medium to wash the cells. If the isolated cells will be used for RNA or DNA isolation, a medium such as PBS may be suitable.
4. Gently pipette the solution into a 10X volume of culture medium.
5. Centrifuge at 300 x g for 7 - 10 minutes to recover cells. Wash cells with culture medium twice prior to use, to ensure Collagenase Type I (0.25%) and collagen are effectively removed.

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