

## Triple Enzyme Mouse Tumor Digestion

Section of Cancer Genomics, Genetics Branch, NCI  
National Institutes of Health

### Reagents and Supplies

**Collagenase, type IV**

Sigma, Cat. C5138

1 g/100 ml = 10X HBSS Stock

**DNase, Type IV**

Sigma, Cat. D5025

20,000 Units/100 ml HBSS = 10X Stock

**Falcon tube, 50 ml**

BD Falcon, Cat. 35-2070

**Fungizone Antimycotic**

Lyophilized, 20 ml

Invitrogen Corp., Cat. 15295017

**Hank's Balanced Salt Solution (HBSS)**

HBSS, without Calcium Chloride or Magnesium Chloride

Mediatech, Cat. 21-022-CV (1-800-235-5476)

**Hyaluronidase, Type V**

Sigma, Cat. H6254

100 mg/100 ml HBSS = 10X Stock

**Nylon mesh filter unit, 70 µm**

BD Falcon, Cat. 35-2350

**Petri Dishes**

Disposable Sterile Petri Dishes 100 x 15 mm

Falcon, Cat. 351029; VWR, Cat. 25373-100

**Tissue culture flasks, 50 ml**

50 ml, Non-vented; T-25

### Preparation

**10X Triple Enzyme Stock Solution:**

Collagenase	1 g	f.c. [10 mg/ml]
Hyaluronidase	100 mg	f.c. [1 mg/ml]
DNase	20,000 Units	f.c. [200 mg/ml]
HBSS	100 ml	

Sterile filter (0.22 µm) and store 5 ml aliquots at -20°C.

Thaw at **RT** (NOT 37°C) before use.

## Establishment of Primary Cultures

1. Remove tumor and place into a 100 mm petri dish and add 5-10 ml HBSS (Hank's balanced salt solution).
2. Quickly mince tumor with scalpels into fragments small enough to be pulled into a 5 ml pipette without getting stuck.
3. Transfer to 50 ml non-vented tissue culture flask.
4. Rinse petri dish with up to 40 ml HBSS and transfer to flask. Total volume in flask should be 45 ml (if not, bring up to volume with HBSS).
5. Add 5 ml 10X Triple Enzyme Mix to the flask, add a stir bar, and incubate at RT on stir plate for 1-3 hr.
6. Pipet up and down to further dissociate cells and pass through 70  $\mu$ m nylon mesh filter unit into a 50 ml Falcon tube (NOTE: filter unit doesn't fit well into tubes from other companies).
7. Pellet cells at 1200 rpm for 8 min.
8. Wash cells 2-3 x in 14 ml HBSS.
9. Resuspend with appropriate volume of plating media and transfer to culture vessel (6 cm<sup>2</sup> plate or T-25 flask).