



Jun 17, 2024

## Dissociation of EBs using Worthington Kit

DOI

<https://dx.doi.org/10.17504/protocols.io.j8nlk8dbxl5r/v1>

Niraj Sawarkar<sup>1</sup>, Regine Tapon<sup>2</sup>, Gist Croft<sup>2</sup>

<sup>1</sup>NYSCF; <sup>2</sup>New York Stem Cell Foundation



Gist Croft

n/a

### Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



**DOI:** <https://dx.doi.org/10.17504/protocols.io.j8nlk8dbxl5r/v1>

**Protocol Citation:** Niraj Sawarkar, Regine Tapon, Gist Croft 2024. Dissociation of EBs using Worthington Kit. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.j8nlk8dbxl5r/v1>

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

We use this protocol and it's working

**Created:** June 17, 2024



**Last Modified:** February 20, 2026

**Protocol Integer ID:** 101973

**Keywords:** ASAPCRN, worthington kit dissociation of eb, dissociation of eb, using worthington kit dissociation, dissociation, eb

**Funders Acknowledgements:**

ASAP

Grant ID: ASAP-000472

## Abstract

Dissociation of EBs using papain



## Reagent prep

- 1 Add 32 ml of EBSS to the albumin ovomucoid inhibitor pipet to dissolve
- 2 Add 5 ml of EBSS to a papain vial. Place in a 37°C water bath for 10-15 min until the papain is completely dissolved and the solution appears clear. Use freshly made papain for each dissociation.
- 3 Add 500 µls of EBSS to a DNase vial. Mix gently – DNase is sensitive to shear denaturation.
- 4 Thaw 1 aliquot of 10mg/ml DNase
- 5 Bring CMF-PBS + Glucose (1 X PBS  $\text{Ca}^{2+}/\text{Mg}^{2+}$  Free) + 12.5 ml of 1M Glucose (Ratio of 1:40)
- 6 Bring CTWM to the hood (1 X PBS  $\text{Ca}^{2+}/\text{Mg}^{2+}$  Free) + 12.5 ml of 1M Glucose (Ratio of 1:40) + 12.5ml of 4% dialyzed BSA (Ratio 1:40) + 5ml of N2 + 10ml B27 + 1 ml of  $\text{MgCl}_2$  (stock 2mM; Ratio 1:500) + 500ml of EDTA
- 7 Prepare 50ml of CTWM plus ODD mix [1X (15ml) CTWM+ODD 2X (30ml)]+Worthington DNase 300ul (10mg/ml DNase)

## Dissociation protocol (optimized)

- 8 Collect EBs and add to a 15ml tube,
- 9 Allow to settle (2 min) if required, spin at 30g for 3 min, and aspirate medium (careful not to aspirate EBs!)
- 10 Add 10m of CMF-PBS+Glucose; allow EBs to settle and aspirate medium



- 11 Prepare Papain DNase solution for all samples by adding 100ul DNase/ 1ml Papain
- 12 Add 1ml Papain/DNase to / ~10M cells or ensure that all the EBs are completely submerged within the Papain/DNase mixture.
- 13 Incubate the vial containing the tissue at 37°C with periodic swirling (a rocker platform is ideal) for 30 to 90 min, depending on age and size of Ebs (day 20 Ebs for 30 min or less, day 33-120 for 90 min). Keep shaking the tube every 15 to 20 minutes to ensure uniform dissociation.
- 14 After incubation is complete, spray the tube with ethanol and return it to the hood.
- 15 Add 8 ml CTWM (Complete Trituration Wash medium)+ODD mixture—
- 16 Spin 300G x 3min
- 17 Aspirate supernatant
- 18 Resuspend with 1ml of CTWM+ODD and Triturate 20 passes with p1000
- 19 Add 9 ml CTWM+ODD and mix
- 20 Important if the triturated EBs start looking sticky and/or thread-like, quickly add 10-15 ul of DNase to the tube and triturate until a homogeneous mixture is obtained.
- 21 Spin 500G x 3min and aspirate supernatant
- 22 If there are still suspended particles throughout the media, add 10-15 ul of DNase and resuspend, followed by spin once again.
- 23 Add 10 ml of seeding media and aliquot to count cells.



- 24 Spin 500Gx3min
- 25 Aspirate sup and resuspend to high-density stock in seeding medium (10M cells/ml, 5M cells/ml, 2M cells ml as appropriate for total cell number in
- 26 Dilute for seeding stock
- 27 Seed 96 wp wells at high density