Jun 17, 2024

Dissociation of EBs using Worthington Kit

DOI
dx.doi.org/10.17504/protocols.io.j8nlk8dbxl5r/v1

Niraj Sawarkar[1], Regine Tipon[2], Gist Croft[2]

[1]NYSCF; [2]New York Stem Cell Foundation

ASAP Collaborative Rese…

N Niraj Sawarkar
NYSCF

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.j8nlk8dbxl5r/v1

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

Protocol status: Working
We use this protocol and it's working

Created: June 17, 2024

Last Modified: June 17, 2024

Protocol Integer ID: 101973

Keywords: ASAPCRN

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 mins 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/nl DNASe

5 Bring CMF-PBS + Glucose (1 X PBS $Ca2^{+/}Mg2^{+}$ Free) + 12.5 ml of 1M Glucose (Ratio of 1:40)

6 Bring CTWM to the hood (1 X PBS $Ca2^{+/}Mg2^{+}$ 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 $MgCl_2$ (stock 2mM; Ratio 1:500) + 500ml of EDTA

7 Prepare 50ml of CTWM plus ODD mix [1X (15ml) CTWM+ODD 2X (30ml)]+Worthignton 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