Sep 10, 2020

Freezing/Cell Banking

In 1 collection

Andrea Argouarch[1]

[1]University of California, San Francisco

1 Works for me dx.doi.org/10.17504/protocols.io.8gmhtu6

Neurodegeneration Method Development Community
Tech. support email: ndcn-help@chanzuckerberg.com


Andrea Argouarch
University of California, San Francisco

ABSTRACT

Protocol includes banking dural cells into cryovials for long term storage in liquid nitrogen.

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PROTOCOL CITATION

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COLLECTIONS

Dural Cell Isolation and Culturing - Collection

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Oct 19, 2019

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PROTOCOL INTEGER ID

28909

PARENT PROTOCOLS

Part of collection
Dural Cell Isolation and Culturing - Collection

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Mr. Frosty Freezing Container, 2mL tubes, Nalgene Mr. Frosty Freezing Container for 1-2mL cryogenic tubes, PC, clear w/ blue lid, 1/Cs.	5100-0001	Thermo Fisher
Cryo-Tags	9187-1100	USA Scientific
Trypan Blue Solution, 0.4%	15250061	Thermo Fisher
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
Fetal Bovine Serum	97068-091	Vwr

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NAME	CATALOG #	VENDOR
DMSO Bio-Max, Cell Culture Grade	40470005-2	bioworld
C-Chip™ Disposable Hemacytometers, Improved Neubauer; 50/Pk.	22600100	Thermo Fisher
DPBS, no calcium, no magnesium	14190250	Thermo Fisher
DMEM, high glucose, pyruvate	11995073	Thermo Fisher
Trypsin-EDTA (0.05%), phenol red	25300062	Thermo Fisher

Observations

1 At 100% confluency, take pictures of cell morphology, freeze down for banking (~0.5 Million per vial), collect remaining cells in flask for gDNA, and seed cells into 1xT25 for karyotyping (~30,000 cells, 60 ul)

2 Turn off UV lights and clean hood with 70% ethanol

3 Clean items with 70% ethanol and bring into hood

a. DPBS -/-

DPBS, no calcium, no magnesium
by Thermo Fisher
Catalog #: 14190250

b. Sterile Filtered Media

DMEM, high glucose, pyruvate
by Thermo Fisher
Catalog #: 11995073

Penicillin-Streptomycin
by Gibco - Thermo Fisher
Catalog #: 15140122

Fetal Bovine Serum
by Vwr
Catalog #: 97068-091

c. Label 2xT150 flasks with ID, date, and p2, add 28 mls **28 mL** of media per flask

d. 0.05% Trypsin

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Trypsin-EDTA (0.05%), phenol red
by Thermo Fisher
Catalog #: 25300062

Preparation

4 Turn off UV lights and clean hood with 70% ethanol

5 Labeling cryovials
a. Create labels using Microsoft Word, following the formatting instructions

Cryo-Tags
by USA Scientific
Catalog #: 9187-1100

b. Print out estimated number of labels for the both flasks (~15) with ID, date, p2, and ~0.5 mill cells.
c. Arrange cryovials in 96, 1.5ml tube holder with spaces in between each vial
d. Spray label sheets thoroughly with 70% ethanol, dry, and bring into hood to adhere onto each vial
e. Label top of each vial with ID
f. Unscrew cap in order to easily lift up when pipetting in cell suspension

6 Clean items with 70% ethanol and bring into hood
a. DPBS $^{-/-}$

DPBS, no calcium, no magnesium
by Thermo Fisher
Catalog #: 14190250

b. Sterile Filtered Media

DMEM, high glucose, pyruvate
by Thermo Fisher
Catalog #: 11995073

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Penicillin-Streptomycin
by Gibco - Thermo Fisher
Catalog #: 15140122

Fetal Bovine Serum
by Vwr
Catalog #: 97068-091

c. 0.05% Trypsin
i. Aliquot to and warm before use

Trypsin-EDTA (0.05%), phenol red
by Thermo Fisher
Catalog #: 25300062

d. Freezing media (made fresh)
i. 100% DMSO to 10%

DMSO Bio-Max, Cell Culture Grade
by bioworld
Catalog #: 40470005-2

ii. 90% Media
e. Incyto disposable hemocytometers

C-Chip™ Disposable Hemacytometers, Improved Neubauer; 50/Pk.
by Thermo Fisher
Catalog #: 22600100

f. Trypan blue, **add** 40 ul **40 µl** into a 1.5 ml tube

Trypan Blue Solution, 0.4%
by Thermo Fisher
Catalog #: 15250061

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g. T25 labeled to send to Cell Line Genetics for karyotyping (KT)

h. 15 ml conical with 10 mls **10 mL** of media to dilute KT cells

i. 15 ml conical to collect cells for gDNA

Culturing

7 Take 3-4 BF images of morphology. Save TIFF under ID and passage number

8 Aspirate old media

9 Rinse by adding 5 mls **5 mL** of DPBS per flask and gently swirling

10 Aspirate DPBS

11 Add 3 ml **3 mL** of trypsin per flask and place in incubator **37 °C** for 3 mins **00:03:00** until cells are detached

a. Can also gently tap the side of the flask to detach cells, check under microscope to confirm

12 Add 9 mls **9 mL** of fibroblast media per flask to inactivate trypsin

13 Collect cell suspension in a 50 ml conical

14 Rinse flask with 12 mls **12 mL** of PBS to collect remaining cells, place in 15ml conical for gDNA

15 Measure final cell suspension volume with serological and mix

16 Dilute 10 ul **10 µl** of cell suspension into trypan blue, tap to mix

17 Load 10 ul **10 µl** of trypan blue and cell dilution into the hemocytometer

18 Count non blue cells in 4 grind corners during spin and then calculate total cells and resuspension volume for 0.5E6 cells per ml

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19 Total cells for 2 flasks = (Cell Count/4 grinds) x 5 dilution factor x 10,000 x ~25mls suspension volume
a. Resuspension volume for 0.5E6 cells per ml = Number of vials

20 Spin both conicals at 1000 rpm **1000 rpm** for 5 mins **00:05:00** during counting

21 Aspirate supernatant for both pellets (freezing and gDNA), being careful to not aspirate the pellet

22 Tap the pellet to resuspend for freezing

23 Store gDNA pellet at -80C **-80 °C** until mycoplasma assay

24 Add freezing media dropwise to cell pellet and add 1 ml **1 mL** per cryovial. Spin KT cell pellet during this time.

25 Prepare for KT in T25 flask
a. Take 60-100 ul **60 µl** of DMSO cell suspension and dilute into 10 mls **10 mL** of media
b. Spin for 1000 rpm **1000 rpm** for 5 mins **00:05:00**
c. Aspirate, resuspend in 5 ml **5 mL** of media, and add to 1xT25 for KT
d. Place flask in incubator (5% CO2, 37^{O}C) **37 °C**
e. Send KT to Cell Line Genetics at 40-50% confluency in about 5-6 days

26 Secure each cryovial cap tightly and place in Mr. Frosty

Mr. Frosty Freezing Container, 2mL tubes, Nalgene Mr. Frosty Freezing Container for 1-2mL cryogenic tubes, PC, clear w/ blue lid, 1/Cs.
by Thermo Fisher
Catalog #: 5100-0001

27 Place Mr. Frosty in -80^{O}C **-80 °C** for 24-48 hours **48:00:00** and then transfer to liquid nitrogen **-190 °C**

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28 Freeze back up cells from 6 well plate, place in Mr. Frosty for 24-48 hours **48:00:00** , then transfer to LN2

-190 °C

Clean Up

29 Throw away biohazard materials properly

30 Clean and sterilize hood with 70% ethanol and turn on UV

31 Update cell culture notes in lab notebook

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