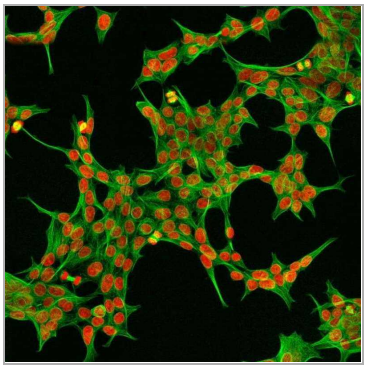

VERSION 1

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We are still developing and optimizing this protocol

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General freezing protocol for HEK-Blue cells V.1

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ABSTRACT

HEK-Blue is a product from Invivogen, which provide reporter cells for endotoxin-testing among others. Here is a generalized protocol for freezing cells with reporter characteristics. This protocol differ significantly from Invivogens recommendations and should be used with care, as it has not been tested on all the different cell lines provided by Invivogen.

GUIDELINES

The protocol has been proven to work with different HEK-Blue cells, but not all. Please follow the recommendations of Invivogen when buying a new batch, and test protocol when a sizable batch of HEK-blue cells are available in storage.

MATERIALS

Centrifuge
Laminar flow cabinet
CO2 incubator

SAFETY WARNINGS

Take care to minimize exposure to DMSO. Use appropriate PPE when working with an ultra-low temperature freezer or vapor-phase nitrogen tank.

BEFORE START INSTRUCTIONS

Prepare freezing medium. Take care to heat and cool the necessary reagents.

Concentrated complete freeze medium (CCMF)

3h 15m

1 Pre-prepare freezing medium. While you could create freeze medium outright, and suspend the

cells directly in it, it is recommended to minimize the exposure time of cells with DMSO. To do this, the cells are initially resuspended and counted in serum-free medium. This give you time to create labels, etc. between resuspension and freezing.

1.1 In 70 mL DMEM, mix 20 mL FBS and 10 mL DMSO 5m

> **Note**
>
> Always use ultra-low endotoxin (>0.1 EU/mL) mediums, FBS and supplements if possible

Materials:

- DMEM high glucose GlutaMAX **Gibco - Thermo Fischer Catalog #31966021**
- ULTRA-LOW ENDOTOXIN FETAL BOVINE SERUM (FBS) **BioWest Catalog #S1860**
- Dimethyl sulfoxide **MP Biomedicals Catalog #196055**

1.2 Aliquot CCMF in 10 mL units and store frozen at -20 °C 10m

> **Note**
>
> Create descriptive labels containing the following information: HEK-Blue 2x freeze medium, DMEM w/GlutaMAX, 20% FBS, 10% DMSO, Date

Freezing procedure 1d 0h 6m

2 When cells reach between 80-90% confluence, the freezing procedure can start

> **Note**
>
> It is important that the cells have at least grown a single passage with selective antibiotics.

> **Note**
>
> It is important to pre-cool and pre-heat certain components, and keep a bucket of ice to minimize loss of viability when adding DMSO.

2.1 Remove supernatant from monolayer. Store 2 mL or more for mycoplasma testing. Wash cell layer gently with PBS. Add 10 mL PBS to a T-75 equivalent flask, and incubate 10m

for 00:05:00. Lightly tap flask, and ensure cell detachment with microscope

Materials:
PBS pH 7.2 **Gibco - Thermo Fischer Catalog #20012019**

2.2 Transfer suspension to a canonical centrifugation tube and centrifuge with 200 rcf, Room temperature, 00:02:00 — 2m

2.3 Decant supernatant and resuspend pellet in 1 mL warm serum-free DMEM — 1m

Materials:
DMEM high glucose GlutaMAX **Gibco - Thermo Fischer Catalog #31966021**

2.4 Create a 100 µL 10x dilution of suspension in PBS, and determine cell concentration. — 10m

Dilute cell suspension in warm serum-free DMEM to 2×10^{6} live cells mL^{-1}. Find volume DMEM to add with v_2 = \frac{1\times c_1}{3\times 10^{6}}, where c_1 is the cell concentration found earlier

2.5 Dispense 500 µL cell suspension to cryotubes or screw cap tubes. Create labels and attach to tubes. Lastly, add 500 µL ice-cold CCFM — 1d

> **Note**
>
> Use cryotubes (Sarstedt #72.379) when freezing for storage in vapor-phase nitrogen tank. Appropriate screw cap tubes (Sarstedt #72.694.217) can be used when cultures are intended for storage in -80 °C

Mix suspension, and transfer tubes to a cold block (Corning #432050) on ice. Transfer tubes or cold block to an insulated box (Corning #432021). Freeze at -80 °C for 24:00:00. Transfer tubes to box, and store long-term in a vapor-phase nitrogen freezer or medium-term in a ultra-low temperature freezer