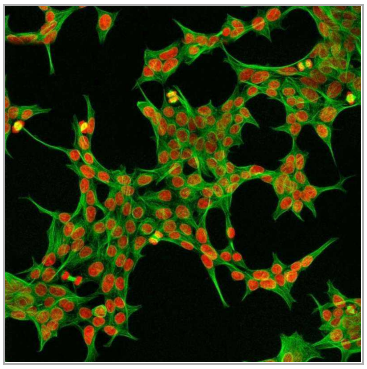

FEB 06, 2023

Protocol status: In development
We are still developing and optimizing this protocol

Created: Jan 25, 2023

Last Modified: Feb 06, 2023

PROTOCOL integer ID: 75836

Keywords: HEK, Endotoxin, TLR, TOLL-Like receptor

General initiation protocol for HEK-Blue cells

Andreas Sagen[1]

[1]The National Institute of Occupational Health in Norway

Andreas Sagen
University of Oslo, The National Institute of Occupational H...

ABSTRACT

HEK-Blue is a product from Invivogen, which provide reporter cells for endotoxin-testing among others. Here is a generalized protocol for initial seeding of cells with reporter characteristics.

GUIDELINES

The protocol has been proven to work with different HEK-Blue cells, but not all. Take time to optimize.

MATERIALS

Centrifuge
Laminar flow cabinet
CO2 incubator

SAFETY WARNINGS

Use appropriate PPE when working with selective antibiotics.

BEFORE START INSTRUCTIONS

Prepare complete initiation medium

Complete initiation medium (CIM)

1 Initially, we want to expand the cells quickly and make them healthy. This require double the amount of FBS and no selection antibiotics in the medium for the first 2-3 passages.

1.1 In 400 mL DMEM, mix 100 mL FBS 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**

1.2 Aliquot CIM in 50 mL units and store frozen at -20 °C

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

Thawing from frozen stock

2 The cells are fragile in the beginning. Take extra good care of the cells when thawing.

2.1 Add 11 mL warm CIM in a T-75 flask and incubate for 00:30:00 in a 5% CO_2 incubator 30m

2.2 Get a vial of HEK-Blue cells, and thaw in a warm water bath for approx. 00:02:00 2m

2.3 Transfer vial content to a centrifuge tube with 5 mL warm CIM

2.4 Centrifuge with 200 rcf, 00:05:00 5m

2.5 Discard supernatant and resuspend pellet in 1 mL warm CIM

2.6 Add all content from centrifugation tube to T-75 and incubate in a 5% CO_2 incubator

> **Note**
>
> Incubate for 2-3 days, then change medium. Passage when cells reach 80-90% confluence

Subculture of early culture 40m

3 The cells are fragile the first 2-3 passages. To maximize growth, it is important to use a special medium formulation and take extra care when working.

3.1 Add 11 mL warm CIM in a T-75 flask and incubate for 00:30:00 in a 5% CO_2 incubator 30m

3.2 Remove supernatant from T-75 and wash with PBS

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

3.3 Add 3 mL PBS and incubate for 00:05:00 5m

3.4 Add 3 mL CIM and transfer content to centrifugation tube

3.5 Centrifuge with 200 rcf, 00:05:00 5m

3.6 Discard supernatant and resuspend pellet in 1 mL warm CIM

3.7 Split between 1:2 to 1:6 of resuspended suspension from centrifugation tube to T-75 and incubate in a 5% CO_2 incubator

> **Note**
>
> Incubate for 2-3 days, then change medium. Passage when cells reach 80-90% confluence

Repeat this procedure once, then change to the general subculture protocol

> **Protocol**
>
> 
>
> NAME
>
> **General subculture protocol for HEK-Blue cells**
>
> CREATED BY
>
> **Andreas Sagen**
>
> PREVIEW