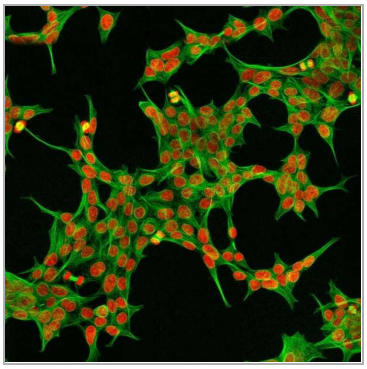

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OPEN ACCESS

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

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General subculture protocol for HEK-Blue cells

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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 culturing 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 growth medium

Complete growth medium (CCM)

3h 15m

1 For the HEK-Blue cells to work correctly, it has to contain a selective agent.

1.1 In 450 mL DMEM, add 50 mL FBS, and selective antibiotics (cell line dependent; Remove equal volume DMEM/FBS):

Zeocin: 0.5 mL
HEK-Blue selection: 2.0 mL
Blasticidin: 1.5 mL

Materials:
DMEM high glucose GlutaMAX **Gibco - Thermo Fischer Catalog #31966021**
ULTRA-LOW ENDOTOXIN FETAL BOVINE SERUM (FBS) **BioWest Catalog #S1860**
Zeocin **InvivoGen Catalog #ant-zn**
HEK-Blue Selection **InvivoGen Catalog #hb-sel**
Blasticidin **InvivoGen Catalog #ant-bl**

1.2 Aliquot complete growth medium in 50 mL or 100 mL units and store refrigerated at 4 °C or frozen at -20 °C

> **Note**
>
> Create descriptive labels containing the following information: Cell line identifier, DMEM, 10% FBS, selective-antibiotic, Date

Subculture process

40m

2 HEK-Blue cells area easy to subculture. They do not require trypsin, only PBS to dissociate from growth surface. Different cell lines of HEK-Blue grow with different doubling times, and the initial seeding density has to be optimized.

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 Remove supernatant from T-75 and wash with PBS

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

2.3 Add 6 mL PBS and incubate for 00:05:00 5m

2.4 Transfer content to centrifugation tube and centrifuge with 200 rcf, 00:05:00 5m

2.5 Discard supernatant and resuspend pellet in 1 mL warm CIM

2.6 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