

[Protocol 1.10] Preparation of skin media and Fib/Hek media for fibroblasts

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Version: 1.0 (01.12.2023)

Media and Reagents:

Skin media:

DMEM High Glucose + GlutaMAX (Life tech. #31966-021)

FBS (Life tech. #16140-071)

Anti-Anti (Sigma #A5955-100ML)

NEAA (Life tech. #11140-035)

FGF (Peprotech #AF-100-18B)

Fib/Hek media:

DMEM High Glucose + GlutaMAX (Life tech. #31966-021)

FBS (Life tech. #16140-071)

MEM Non-essential amino acid solution 10mM (Life tech. #11140-035)

(optional) Pen./Strep. (Life tech. #15140-122)

Materials and Equipment:

50 ml pipettes

100/200/500/1000 ml sterile bottles

1. Introduction and Purpose

This protocol describes how to prepare and skin media and Fib/Hek media.

IMPORTANT:

Skin media is used for thawing fibroblast cells containing more factors to protect the cells from the freeze-thaw stress. After 24 hrs, the skin media will be aspirated and Fib/Hek media will continuously used for cultivating fibroblasts

2. Procedure - Skin media

The recipe is followings:

Materials	Volume (ml)
DMEM High Glucose + GlutaMAX (Life tech. #31966-021)	880
FBS (Life tech. #16140-071)	100
Anti-Anti (Sigma #A5955-100ML)	10
NEAA (Life tech. #11140-035)	10
FGF (Peprotech #AF-100-18B)	50 µl (200 ng/µl stock; final con: 10 ng/ml)

1. The half of the recipe can be prepared in a new DMEM bottle after removing 60 ml of it into a using DMEM bottle, e.g. for coating.
2. Mix well 1 L or 500 ml of this media and label as followings: **Skin media + 1x Anti-Anti + 1x NEAA + 10 % FBS + 10 ng/ml FGF, date of the preparation, your name**
2. Aliquot it as needed and store at 4°C

3. Procedure - Fib/Hek media

The recipe is followings:

Materials	Volume (ml)
DMEM High Glucose + GlutaMAX (Life tech. #31966-021)	440
FBS (Life tech. #16140-071)	50
MEM Non-essential amino acid solution 10mM (Life tech. #11140-035)	5
(optional) Pen./Strep. (Life tech. #15140-122)	5

1. From a new DMEM bottle, remove 60 ml of it into a using DMEM bottle, e.g. for coating and prepare the Fib/Hek media directly in the new bottle.
2. Mix well 500 ml of this media, remove the DMEM label, and label as followings: **Fib/Hek media + 10 % FBS + 1 % MEM (+ 1 % Pen/Strep), date of the preparation, your name**
3. Aliquot it as needed and store at 4°C