

[Protocol 1.05] Geltrex coating of culture vessels

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Protocol 1.05 Geltrex coating of culture vessels
Version: 1.0 (24.01.2022)

Media and Reagents:

Geltrex (Thermo Fisher, A1413302)
KO DMEM/F-12 (Thermo Fisher, 12660-012)
Alternatively: DMEM/F-12, HEPES (Thermo Fisher, 11330032)

Materials and Equipment:

Aspiration Pump
5ml/10ml pipettes
6/12/24 well plates **or**
T25/T75 flasks **or**
IVF-dish
15/50 ml Falcon tube

1. Introduction and Purpose

This protocol describes the procedure for the preparation of Geltrex coated culture vessels for the maintenance of induced pluripotent stem cells (iPSCs).

2. Storage of Geltrex

Store Geltrex stock vials (5 ml) after delivery at -20°C.

Aliquots: Stock vials (5 ml) of Geltrex must be thawed on wet ice overnight in the refrigerator.

Note: The following steps have to occur on ice! All serological pipettes and tubes to be used should be cold: therefore pre-chill at -20°C for at least 20 minutes or alternatively cool the pipettes by pipetting several times with cool media!

Prepare 1:10 dilution of Geltrex (5 ml) in ice-cold KO DMEM/F-12 (45 ml) and aliquot this Geltrex solution in portions of 0.5 ml per vial. These aliquots should be stored at -20°C.

Alternatively: Prepare 0,5 ml aliquots of Geltrex **w/o** KO DMEM/F-12 Media and store at -20°C. *Please communicate with the requester which way to aliquot!*

3.1 Coating procedure

1. Prior to use, remove an appropriate number of Geltrex aliquots from -20°C storage and allow thawing at RT for 2 -5 minutes.

Note: Do not repeatedly freeze and thaw this reagent!

- Mix 0.5 ml Geltrex with 5.5 ml cold KO DMEM/F-12 in a 15 ml falcon tube (1:12 from 1:10 stock; 1:120)
- Add appropriate volume of the diluted Geltrex solution to each well/flask (see table below).
- Incubate the tissue culture vessel with Geltrex for 30 minutes at 37 °C or for 1 hour at RT.
- Aspirate Geltrex from vessel right before use.

3.2. Coating procedure (Alternatively)

1. Prior to use, remove an appropriate number of Geltrex aliquots from -20°C storage and allow thawing on wet ice at 4°C for at least 30 min.

Note: Do not repeatedly freeze and thaw this reagent!

- Mix 0,5ml Geltrex with 49,5 ml cold DMEM/F-12, in a 50 ml falcon tube.
- Add appropriate volume of the diluted Geltrex solution to each well/flask (see table below).
- Incubate the tissue culture vessel with Geltrex for 30 minutes at 37 °C or for 1 hour at RT.
- Aspirate Geltrex from vessel right before use.

Culture Vessel	Surface Area [cm ²]	Volume of diluted Geltrex per vessel/ per well[ml]
96 well	0,32	0,05
24 well	2	0,25
12 well	4	0,5
IVF-dish	~ 2,5	0,5
6 well	10	1,0
T25	25	2,5
T75	75	7,5

Note: The coated plates can also be stored at 2-8 °C sealed with parafilm (make sure coating solution does not dry out!) for up to a week. Prior to use pre-warm the culture vessel to roomtemperature.