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Coating of plates with Geltrex, for human iPSC culture

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We use this protocol and it's working

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Guidelines

Geltrex Catalog NO: A1413302 (5 ml from ThermoFischerScientific). Thin Gel Method (non-gelling) for hESC Applications. From: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3157883/

Safety warnings

When working with volumes of Geltrex <5ml, aliquot to appropriate required working volumes and store at -20 to -80°C. Avoid multiple freeze/thaw cycles.

Before start

Thaw GeltrexTM Basement Membrane Matrix at 2-8°C overnight. Refrigerator temperatures may vary; therefore, thaw extract on ice in a refrigerator. Basement Membrane Matrix gels in 5-10 minutes above 15°C; therefore when working from a full 5 ml vial, it is unnecessary to keep it on ice if used within 5 minutes and the environmental temperature does not exceed 25°C. It is also unnecessary to prechill pipette tips, tubes, plates, or other objects that may come in contact with the extract. Since smaller volumes warm more quickly, partial tubes and aliquots should be kept on ice to prevent premature gelling.

Thawing of Geltrex, dilution in DMEM/F12 and aliquotting for storage at -20C

1 4 °C

Thaw one tube of Geltrex (1-5 mL) slowly at 2-8°C.

Prepare 20X 1.5 ml Eppendorfs for aliquotting of Geltrex, label: 50X

Mix Geltrex by slowly pipetting solution up and down; be careful not to introduce air bubbles.

On ice: in a 15 ml Falcon tube, mix Geltrex 1:1 with **cold** DMEM/F12 (Sigma #D6421) and aliquot at 0.5 ml.

Store the aliquots at -20C.

(REMEMBER TO CHECK EXPIRY DATE ON EVERYTHING)

Plate coating

2 Take plates that require coating and label.

Thaw an aliquot of Geltrex/DMEM/F12 (1:1 mix) on ice.

Prepare an aliquot of 22 ml cold DMEM/F12 = keep on ice. 4 °C

On ice: Dilute 450 ul of 1:1 Geltrex (50X) ~1:50 into 22 ml **cold** DMEM/F12 medium for a final dilution of 1:100. Empirical determination of the optimal coating concentration for your application may be required. Volumes can be adjusted accordingly.

Add a sufficient amount of diluted Geltrex solution to cover the entire area onto growth surface (100 ul/well in 96-well plate; 0.5 ml/well for 24-well and 4-well Ibidi imaging dish; 1 ml for 35 mm dish, 3 ml for 60 mm dish, i.e. 0.14 ml/cm2 → 1 ml per 6-well dish; 8 ml per T75 flask).

Coat the dish and place in incubator (37C) for 60 min (no need to seal). 37 °C

Afterwards, take the plates that you will need for cell seeding out to room temperature for minimum 30 min. Store the remaining plates at 4C for up to 2 weeks (best for only 1 week to be on the safe side; parafilm to avoid drying out).

Do not allow coated surface to dry out and maintain a storage temperature of 2 to 8°C to avoid premature gelling. 4 °C

At time of use, aspirate GeltrexTM coating and immediately plate cells in pre-equilibrated cell culture medium. No rinsing!