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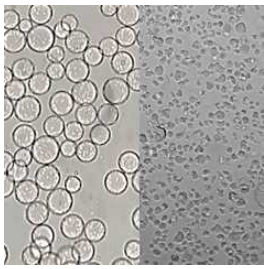
Harvest of adherend cells from Cytodex® 1 beads



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Protocol status: Working
We use this protocol and it's working

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Abstract

Expanding adherend cells to quanteties suitable for cell therapy is a subject of increasing relevance.
Variouse cell types either autolog or allogen - could in future be used for medical purposes / in clinical application as a key component in therapy. This protocol discribes how to harvest adherend cells from dextran based Cytodex® 1 beads after expansion. Since other enzymatic schemes like Trypsin-EDTA or TrypLE^{TM} have shown low efficiency and yield - here we present an approach that is easy in implementation and delivers cells with high harvesting yield and high viability.

Guidelines

This protocol can surely be further improved but besides that is pretty much strait forward - crutial steps are the centrifugation and supernatent discartion where many cells can be lost if samples are not handled with due care.

Materials

DMEM - 4.5g/L glucose - stable glutamine - sodium pyrovate - Biowest [L0103-500]
10% FBS - Corning
1x P/S - Corning

PBS

10x TrypLE Express - Gibco

Dextranase Plus L, 1,6-α-D-Glucan-6-glucanohydrolase from Chaetomium erraticum
Novozymes - >100 KDU-A/G [Dextranase units]

Cytiva - Cytodex1 beads [17044803]

Clean bench
Incubator - 37°C - 5% CO2
Centrifuge
Centrifugation tubes 15/50mL
Eppendorf tubes 1.5mL
Tube rotator
Pipetboy + pipets [5-50mL]
Pipets - [10-1000uL]
NucleoCounter NC-200 + Solution A100 and B
Neubauer Chamber
Vacuum pump

Spinner Flask - Pfeiffer 500mL
Magnetic stirrer - Technomara

adherent primary cells - MSC / Chondrocytes / HUVEC / Fibroblasts

Troubleshooting

Safety warnings

There are no particular safety risks exceeding the risk during cell culture.

sampling

1h 26m

1 withdraw homogenized cell solution from culture vessel and transfer to centrifuge tube 10-50 mL

1.1 take a sample from each tube for counting to determine available cell number

Micro carrier solving

1h 26m

2 add [M] 0.1 % volume of dextranase (v/v)

3 transfer samples to a tube rotator and place in an incubator 10 rpm, 37°C, 01:00:00 1h

4 take out tubes and centrifuge 600 x g, 00:08:00 , acc 9 - dec 5 8m

5 carefully discard supernatant

single cells

1h 26m

6 add a small volume 1-5 mL of 2x TrypLE™ and completely solve the pallet

6.1 add more 9-45 mL of 2x TrypLE™, transfer tubes to a Tube rotator and place in an incubator 10 rpm, 37°C, 00:10:00 10m

7 take out tubes and centrifuge 600 x g, 00:08:00 , acc 9 - dec 5 8m

8 discard supernatent and solve pallet in adequat amount of culture medium 1-5 mL

8.1 filtrate cell solution through a 40 µm Filter to exclude micro carrier fragments

Yield, viability and follow up

9 count cells and determine viability

10 use cells for further analysis