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Huh7.5_SOP

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1 Works for me dx.doi.org/10.17504/protocols.io.biapkadn

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ABSTRACT

SOP for thawing, cultivation and freezing of the Huh7.5 cell line by the Division Systems Biology of Signal Transduction, German Cancer Research Center (DKFZ).

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KEYWORDS

Huh7.5 cell lines, cell culture, medium, thawing, freezing

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MATERIALS

NAME	CATALOG #	VENDOR
Fetal Bovine Serum	10270106	Gibco - Thermo Fischer
Neubauer Improved (NI) Hemocytometer	22-600- 100	Life Technologies
0.05% Trypsin-EDTA, phenol red	25300054	Invitrogen - Thermo Fisher
Glutamax (100x)	35050-061	Gibco - Thermo Fischer
DMEM, high glucose, no glutamine, no phenol red	31053028	Thermo Fisher
Bovine Serum Albumin	A9418	Sigma Aldrich
Penicillin-streptomycin (P/S)	15140122	Gibco - Thermo Fisher
Dimetylsulfoxide – DMSO	#41639-100ML	Merck Millipore Sigma
DPBS w/o: Ca and Mg	P04-36500	PAN Biotech
15 ml centrifuge tubes	188271	greiner bio-one
50 ml centrifuge tubes	227261	greiner bio-one
Tissue Culture Dish D:150 mm	93150	Techno Plastic Products (tpp)
Tissue Culture Test Plates 6-well	92006	Techno Plastic Products (tpp)

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NAME	CATALOG #	VENDOR
CRYO.S 2 ML PP ROUND BOTTOM INTERNALTHREAD	122263-2DG	greiner bio-one
Cool Cell LX	210004	Biozym
Mr. Frosty	5100-0001	Thermo Fisher Scientific
Trypan Blue solution 0.4% for microscopy	935395	Merck Millipore Sigma

Cells

1 Huh7.5 cells were kindly provided by Charles M. Rice (The Rockefeller University, NY, RRID:CVCL_7927)

1.1 Cells are cultivated in incubator at 37°C and 5 % CO_2 incubation and 95 % relative humidity

Medium

2 **Growth Medium**
DMEM
1% Glutamax
10% FCS
1% P/S

3 **Growth factor-depleted Medium**
DMEM
1% Glutamax
1% P/S
1 mg/ml BSA

4 **Freezing Medium**
70% Growth-Medium
20% FCS
10% DMSO

Thawing of cells

5 Pre-warm growth medium in **37 °C** water bath

6 Thaw the cells in a **37 °C** water bath (not completely, there should be a small visible ice clump inside the tube)

7 Transfer the cells into a 15 ml centrifuge tube containing 9 ml pre-warmed growth medium

7.1 To this aim, use 1000 µl pipette and add some medium to cryotube, and mix. With this step the little ice clump should disappear

8 Transfer all liquid from the cryotube to the centrifuge tube, rinse cryotube again and transfer to centrifuge tube

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9 **1000 rpm, Room temperature 00:03:00 , 130 x g**

10 Aspirate supernatant

11 Resuspend cells in 10 ml fresh growth medium (~7 times)

12 Transfer the cells to a 150-mm dish (~2x10^6) in 25 ml growth medium

13 Let cells adhere to the surface of the dish over night in incubator

14 Replace the medium with 25 ml fresh growth medium the next day

15 Split after 2-3 days as described in "Cultivation of cells" (check for confluency to be around 80% to 90%; for example: thawn on Thursday, change medium on Friday and split on Monday)

Cultivation of cells

16 Cells are used for experiments until passage 25 (total passage number starting from the stocks provided by Charles M. Rice, The Rockefeller University)

17 Cells are split every 3 to 4 days (e.g. Monday to Friday and Friday to Monday)

18 On one ~80-90% confluent 150-mm plate, 7-10x10^6 cells can be expected

19 Aspirate the growth medium

20 Wash the cells with 10 ml DPBS once

21 Detach the cells from surface by adding 0.05% Trypsin-EDTA (3 ml for 150-mm dish)

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22 Place the cells with Trypsin-EDTA in an incubator and incubate for 3 to 4 minutes by which the cells should be detached from the surface

23 Tap the dish carefully and stop the enzyme reaction of Trypsin-EDTA by adding growth medium containing FCS (7 ml for 150-mm dish)

24 Resuspend the cells and transfer to centrifuge tube

25 **1000 rpm, Room temperature 00:03:00 , 130 x g**

26 Aspirate supernatant

27 Resuspend in 10 ml fresh growth medium (~7 times) to remove all clumps

28 Dilute 50 µl of cells 1:2 using medium, count the cells applying a 1:2 dilution with Trypan blue using a Neubauer improved hemocytometer

29 Plate the cells as required for your planned experiment or for maintaining the cell culture

29.1 Keeping cells in culture:
4 days: $2x10^6$ cells in 25 ml growth medium / 150-mm dish
3 days: $3x10^6$ cells in 25 ml growth medium / 150-mm dish

29.2 For experiments: usually $0.6x10^6$ cells / well of a 6-well plate in 1.5 ml of medium is used

29.3 After 24 h, wash cells 3 times with DPBS , add 1.5 ml of growth-factor depleted medium and incubate for 3 h

29.4 Stimulate cells and perform experiment

Freezing of cells

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30 Count the cells as described above **go to step #19**

31 Transfer cell suspension to centrifuge tube

32 **1000 rpm, Room temperature 00:03:00 , 130 x g**

33 Label cryotubes (name of cell line, number of cells, passage, date and your initials)

34 Resuspend cells to a density of $2x10^6$ cells / ml in freezing medium

34.1 Calculate needed amount of freezing medium (E.g. if freezing $6x10^6$ cells, 3 ml are needed)

34.2 Prepare freezing medium, mix well

34.3 Resuspend pellet in freezing medium using a serological pipette and transfer to cryotubes (1 ml/tube)

35 Transfer cryotube to Mr. Frosty or Cool Cell LX, only use room temperature Mr. Frosty or Cool Cell LX, never cold ones

36 Transfer cells in cryotubes in Mr. Frosty or Cool Cell LX to -80°C

37 Transfer tubes to liquid nitrogen tank on the next day

38 Record name of cell line, number of cells, passage, date, your name and location of the tube in liquid nitrogen tank

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