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Protocol for the growth and maintenance of mammalian cell lines

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We use this protocol and it's working

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Abstract

This protocol details about the growth and maintenance of mammalian cell lines.

Materials

Phosphate Buffered Saline (PBS) buffer

	A	B	C	D
	Reagents	1 liter of 1X	4 liter of 10X	Final concentration (mM)
	Potassium Phosphate monobasic (KH2PO4)	0.144g	5.76g	1.1 mM
	Sodium chloride (NaCl)	9g	360g	155.2
	Sodium Phosphate dibasic (Na2HPO4)	0.421g	16.842g	3

The following protocol was written for HeLa cells but can be adapted to many other standard mammalian cell lines. 15m

1 Remove 1x PBS, trypsin, and media (DMEM+10%FBS+1%pen/strep supplement (Gibco) from the fridge and warm it up in a 37 °C water bath for 00:10:00 . 10m

2 Remove containers from water bath and disinfect with 70% EtOH before placing in cell culture hood.

3 Take the existing cells in the 75 undetermined ^{2} cell culture flask in the incubator and place under hood after observing under microscope to ensure that cells are near confluent.

4 Attach a sterile Pasteur pipette to the vacuum tube so that the media can be aspirated from the flask by tilting the container so that the media is at front facing left corner where the pipette tip is.

5 Wash cells with 10 mL of PBS added to the bottom most surface away from the cells then place flask down horizontally and gently move side to side to rinse the cells.

6 Aspirate again.

7 Add 3 mL of pre-warmed trypsin to cover the cells.

8 Incubate for 00:05:00 or until the cells have released from the flask. 5m

9 Add 5 mL of fresh media.

10 Collect cell suspension from the flask and wash the side possessing the cells several times before transferring solution to 15 ml conical tube.

11 Centrifuge the conical tube for 2.5 minutes at 100 x g .

12 Aspirate to leave behind just the cell pellet and a little bit of media to cover the cell pellet.

13 Resuspend in 10 mL of media and pipette up and down to disperse cell clumps. Avoid the formation of bubbles.

14 Obtain a new flask and place 12 mL of fresh media into it and before transferring 1 mL (1:10) of the cell suspension into it. Or alternatively distribute the desired number of cells (counted with hemocytometer or other cell counting device) to the appropriate cell culture dish/flask for downstream experiments.

Phosphate Buffered Saline (PBS) buffer

15

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16 Place 3.5 L Milli-Q water in a 4L beaker and add chemicals. Stir on a stir plate until dissolved.

17 Once dissolved, check pH and adjust to pH 7.4 .

18 Pour the solution into a 4L graduated cylinder and adjust volume to 4 L with water.

PBS for Cell Culture

40m

19 Make 2 L of 1x PBS pH 7.4 : 200 mL of 10x PBS pH 7.4 + 1800 mL of H_2O

20 Decant into 250 ml bottles and autoclave 00:40:00 on liquid cycle. 40m