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Recommended Protocol for HEK293 CDM15 Medium Adaption, Cell Culture and and protein expression, lentivirus, adenovirus, adeno-associated virus expression operation instructions

HEK293 CDM15细胞培养基适应、细胞培养及用于蛋白表达，慢病毒、腺病毒、腺相关病毒表达操作说明

1. Introduction 简介

The document is to describe the HEK293 (293F, 293T, 293H, Expi293) medium adaption, cell passage and scale-up, cell cryopreservation, cell thawing, and protein expression, lentivirus, adenovirus, adeno-associated virus expression operation instructions in TPP tubes or shaker flasks using Media.

本文件描述了HEK293（293F、293T、293H、Expi293）等细胞在TPP管或摇瓶中的培养基的适应、细胞传代和扩增、细胞冻存、细胞复苏及用于蛋白表达，慢病毒、腺病毒、腺相关病毒表达。

2. Cell Culture Protocol 细胞培养操作

2.1 Standard Parameters 标准参数

Parameter	Value
50 mL TPP Tube	Culture Volume: 10 ~ 30 mL
125 mL Shaker	Culture Volume: 15 ~ 40 mL
250 mL Shaker	Culture Volume: 40 ~ 80 mL
500 mL Shaker	Culture Volume: 100 ~ 200 mL
1000 mL Shaker	Culture Volume: 200 ~ 300 mL
Shaking Speed	For TPP Tube: 200rpm @50mm diameter of orbital throw shaker; For Shaker Flask: 150rpm @25mm diameter of orbital throw shaker; 90 ~ 120rpm @50mm diameter of orbital throw shaker;
Culture Medium	HEK293 CDM15
Seeding Density	0.5×10^6 cells/mL
Incubation Temperature	37°C
Incubation CO2 Concentration	5%
Incubation Relative Humidity	80% RH

2.2. Cell Culture Medium Adaption 培养基的适应

The HEK293 cells adaptation is performed by transferring cells grown in other medium to a new medium consisting of 50% original medium and 50% medium (HEK293 CDM15).

在原用培养基中生长的HEK293细胞需要转移到由50%原用培养基和50%培养基（HEK293 CDM15）组成的新培养基中进行适应性生长。

2.2.1 On the day of cell passage, sample 0.5 mL of cell suspension and analyze the viable cell density ($\times 10^6$cells/mL) and viability (%) of the sample using cell counter equipment. Transfer the cells grown in original medium into two new media (50% HEK293 CDM15+50% original medium) using a seeding density of 0.5×10^6 viable cells/mL, then incubate cells at the specified environmental conditions (see Section 2.1).

细胞传代当天，取0.5mL细胞悬液，用细胞计数仪分析活细胞密度（$\times 10^6$细胞/mL）和细胞活率（%）。将原用培养基中生长的细胞移入新培养基（50% HEK293 CDM15 + 50%原用培养基），接种密度为0.5×10^6/mL，传代后按照规定的环境条件培养细胞（见第2.1节）。

2.2.2 After 72 ± 3 hours, repeat section 2.2.1. The HEK293 cells need to be adapted in 3 passages. The final growth medium was HEK293 CDM15, depending on the cell growth state (Viable cell density and Viability). Chose HEK293 CDM15 medium as the final growth medium, the operational steps were 2.2.3~2.2.4.

细胞培养至72 ± 3小时后，重复2.2.1步骤，建议按此方法进行3次细胞适应性传代。根据细胞生长状态（活细胞密度和活率），选择HEK293 CDM15 培养基为最终培养基，操作步骤为2.2.3~2.2.4。

2.2.3 If the cell grow well in the medium which contains 50% HEK293 CDM15 medium. After 72 ± 3 hours, sample 0.5 mL of cell suspension and analyze the viable cell density ($\times 10^6$cells/mL) and viability (%) of the sample using cell counter equipment. Transfer the cells into fresh HEK293 CDM15 medium using a seeding density of 0.5×10^6 viable cells/mL and the culture conditions seeing section 2.1.

如果细胞在含有50%HEK293 CDM15 培养基的培养基中生长良好，在培养的72 ± 3小时，取0.5mL细胞悬液，用细胞计数仪分析活细胞密度（$\times 10^6$细胞/mL）和细胞活率（%），将细胞转移至新鲜的HEK293 CDM15培养基中，接种密度为0.5×10^6/mL（见第2.1节）。

2.2.4 After 72 ± 3 hours, repeat section 2.2.3. The HEK293 cells need to be adapted 3 passages before viral infection.

细胞培养至72 ± 3小时后，重复第2.2.3节，建议按此方法进行3次细胞适应性传代，方可进行病毒接种实验。

* Pre-warm all the media to 37°C prior to use.

*培养基在使用前37°C预热。

2.3. Cell Passage And Scale-Up 细胞传代和扩增

2.3.1 Warm completed medium (HEK293 CDM15) to 37°C in a water bath.
37°C水浴中预热HEK293 CDM15 培养基。

2.3.2 Clean/disinfect the biosafety cabinet (BSC) as appropriate with 75% alcohol.
使用75%酒精清洁/消毒生物安全柜。

2.3.3 Spray the outside of the medium bottle with 75% alcohol equivalent and place into the BSC.
使用75%酒精喷洒培养基瓶，并置于生物安全柜。

2.3.4 Remove the culture flask(s) (or TPP) containing growing cells from the incubator. Spray the outside of the culture flask(s) with 75% alcohol and place into the BSC.
从培养箱中取出摇瓶（或TPP管），喷洒75%酒精并置于生物安全柜。

2.3.5 Aseptically sample 0.5 mL of cell suspension and determine the viable cell density ($\times 10^6$ cells/mL) and viability (%) of the culture(s) by cell counter equipment.
取0.5mL细胞悬液，用细胞计数仪分析活细胞密度（$\times 10^6$细胞/mL）和细胞活率（%）。

2.3.6 If the cell density is less than 2.0×10^6 viable cells/mL or the viability is lower than 80% before passaging, the cells need to be centrifuged at 150g ~ 300g (approx. 800 rpm to 1200 rpm) for 5 minutes. Carefully remove the spent media, then resuspend cells with 100% fresh media using a seeding density of 0.5×10^6 viable cells/mL. Seed the cells into a new flask(s) (or TPP), and incubate cells at the specified environmental conditions (see Section 2.1).
传代前，如果细胞密度低于2.0×10^6/mL，或细胞活率低于80%，需要150g ~ 300g（大约800 rpm~1200 rpm）离心5分钟处理细胞。轻轻弃上清，使用100%新鲜培养基按0.5×10^6/mL重悬细胞，并将细胞接种至新摇瓶（或TPP管），传代后按照规定的环境条件培养细胞（见第2.1节）。

2.3.7 If the cell density is more than 2.0×10^6 viable cells/mL and viability is higher than 85% before passaging, transfer an appropriate amount of cell suspension into a new flask(s) (or TPP) and adjust the final culture volume with fresh media for cell passaging directly, then incubate cells at the specified environmental conditions (see Section 2.1).
如果传代前细胞密度大于2.0×10^6/mL且活细胞率高于85%，则将适量的细胞悬液转移到新摇瓶（或TPP管）中，并用新鲜培养基直接调整最终培养体积进行细胞传代，然后在规定的环境条件下培养细胞（见第2.1节）。

*This means the cells need to be centrifuge down and re-suspended in 100% fresh media if the split ratio (seeding density after passage: cell density before passage) is > 1:4.
*这意味着如果传代比（传代后的种子密度：传代前的细胞密度）大于1:4，细胞需要离心并重新悬浮在100%新鲜培养基中。

2.4. Cell Cryopreservation 细胞冻存

2.4.1 Before cell freezing, put the cooling box (add fresh isopropanol in the box), freeze storage tube and other required materials into the refrigerator at 4°C, and precool them for at least 24 hours

before cryopreservation, or put them at -20°C for 2 h~4 h before cryopreservation.

细胞冷冻前，将含新鲜异丙醇的程序降温盒、冻存管等所需材料置于4°C冰箱中，预冷至少24小时后冷冻保存，或在-20°C下放置2小时~4小时后冷冻保存。

2.4.2 Take sample, detected cell density (×10^6cells/mL) and viability (%).

取样计数，检测分析活细胞密度（×10^6细胞/mL）和细胞活率（%）

2.4.3 Prepare the cell freeze solution containing 20% DMSO, and place it on ice after preparation, it's better place in 4°C pre-cooling for 1 h ~ 2 h.

制备含20%DMSO的细胞冷冻液，制备后置于冰上，以4°C预冷1h ~ 2h为宜。

Frozen media: 20% DMSO+80% HEK293 CDM15

冻存培养基：20% DMSO+80% HEK293 CDM15

2.4.4 According to the frozen number, volume and density of frozen cells (the recommended freezing density is 10 ~ 20×10^6cells/mL /vials), calculate the amount of cells needed, and transfer them to a centrifuge tube for centrifugation at 800 rpm ~ 1500 rpm (i.e. 150g ~ 300g) for 5 min.

根据冻存细胞的数量、冻存体积和冻存密度（建议冻存密度为10 ~ 20×10^6 /mL/支），计算所需的细胞量，并移入离心管中，以800 rpm ~ 1500 rpm（即150 g ~ 300 g）离心5min。

2.4.5 Remove the supernatant, pat the bottom of the centrifuge tube to evenly disperse the cells, add an growth media with half the frozen volume, and gently blow the cells to produce a uniform cell suspension; Refer to the table below (Example and according the passage data which medium you choose):

弃上清液，拍打离心管底部使细胞均匀分散，加入冻存体积一半的生长培养基，轻轻吹打细胞，形成均匀的细胞悬液，见下表（示例及根据传代数据选择哪种培养基）：

Media	Composition	Volume	Complete media
Frozen media	20% DMSO+80% HEK293 CDM15	10mL	10% DMSO+90% HEK293 CDM15
Growth media	HEK293 CDM15	10mL	

2.4.6 Use a sterile pipette to transfer the frozen media of the same volume (Containing 20%DMSO) and add it to the cell suspension drop by drop. While adding the frozen media, gently shake the centrifuge tube to maintain the uniformity of cell density.

用无菌移液管转移相同体积的冻存培养基（含20% DMSO），逐滴加入细胞悬液中。加入冻存培养基时，轻轻摇动离心管，保持细胞密度均匀；

2.4.7 Transfer 1 mL ~ 1.5 mL cell freeze suspension with disposable sterile pipette to the pre-cooled freeze tube, which shall be stored on ice.

用一次性无菌移液管将1mL ~ 1.5mL细胞冻悬液移入预冷冻存管中，置于冰上保存；

2.4.8 It is necessary to mix the frozen cell suspension repeatedly during the packaging. After completed the packaging, the frozen tube is transferred to the pre-cooled program cooling

box and stored in the -80°C refrigerator for at least 24 hours.

冻存细胞悬液在分装过程中需要反复混合。分装完成后，将冻存管转移至预冷的程序降温盒中，在-80°C冰箱中至少保存24小时；

2.4.9 Transfer to liquid nitrogen tank for storage.

转移至液氮罐中继续保存。

2.5. Cell Thawing 细胞复苏

2.5.1 Thawing of Vial on Day 0

Day 0时复苏细胞

2.5.2 Clean/disinfect the bio-safety cabinet as appropriate with 75% alcohol.

使用75%酒精清洁/消毒生物安全柜。

2.5.3 Pre-warm the medium (HEK293 CDM15) in 37°C water bath.

37°C水浴中预热HEK293 CDM15 培养基；

2.5.4. Spray the outside of the medium bottle with 75% alcohol and place the bottle into the bio-safety cabinet.

使用75%酒精喷洒培养基瓶身，并置于生物安全柜；

2.5.5 Remove the frozen vial(s) from liquid nitrogen tank and place the vial(s) in dry ice.

从液氮罐中取出冻存管，并置于干冰上；

2.5.6 Thaw one vial at a time in 37°C water bath. Gently agitate the vial within 1 minutes until the ice in the vial melting.

复苏1支细胞，并在37°C水浴中，轻轻晃动冻存管，解冻1分钟；

2.5.7 Spray the outside of the vial with 75% alcohol and place into the bio-safety cabinet.

用75%酒精喷洒冻存管外壁；

2.5.8 Aseptically pipet the contents from the vial gently into a centrifuge tube containing 30 mL of pre-warmed medium (HEK293 CDM15). If necessary, use the pre-warmed medium (HEK293 CDM15) to wash out the contents from the vial.

用无菌移液管将冻存管中的细胞轻轻移到含有30mL预热培养基（HEK293 CDM15）的离心管中。如有必要，使用预热的培养基（HEK293 CDM15）冲洗冻存管中的细胞；

2.5.9 Spin down the cells by centrifugation at 150g ~ 300g (approx. 800 rpm to 1200 rpm) for 5 minutes. Discard the supernatant and re-suspend cells in 10 ~ 30 mL fresh pre-warmed medium (HEK293 CDM15), then adjust the cell density to 0.5×10^6 cells/mL in plastic shaker flask by using pre-warmed medium (HEK293 CDM15).

150g ~ 300g（约800rpm ~ 1200rpm）离心5分钟，丢弃上清液并将细胞重悬于10 ~ 30mL新鲜预热培养基（HEK293 CDM15）中，然后使用预热培养基（HEK293 CDM15）在摇瓶中将细胞密度调节至0.5×10^6cells/mL；

2.5.10 Sample 0.5 mL of cell suspension and analyze the viable cell density ($\times10^6$cells/mL) and viability (%) of the sample using cell counter equipment.

取0.5mL细胞悬液，用细胞计数仪分析活细胞密度（$\times 10^6$/mL）和细胞活率（%）；

2.5.11 If the cell viability is > 85%, incubate the shaker flask in the condition as stated in Section 2.1. Otherwise, consult with manager.

如果细胞活率＞85%，将细胞置于规定的环境条件下培养细胞（见第2.1节）。否则，请咨询相关负责人；

2.5.12 Passage and scale-up the cells.

传代、扩大培养细胞。

2.6. Suspension 293 cell （293F、293T、293H、Expi293）protein expression, lentivirus, adenovirus, adeno-associated virus expression 蛋白表达，慢病毒、腺病毒、腺相关病毒在悬浮293细胞（293F、293T、293H、Expi293）上的表达

2.6.1 Cells growth in HEK293 CDM15

细胞在HEK293 CDM15中培养；

2.6.1.1.After 72 ± 3 hours, sample 0.5 mL of cell suspension and analyze the viable cell density ($\times 10^6$cells/mL) and viability (%) of the sample using cell counter equipment.

72±3 小时后，取 0.5mL 细胞悬液，用细胞计数仪分析活细胞密度（$\times 10^6$/mL）和细胞活率（%）；

2.6.1.2.Dilute the cultures with the same volume of pre-warmed HEK293 CDM15 medium (the volume of cultures: the volume of HEK293 CDM15 medium to be added = 1:1).

用相同体积的预热 HEK293 CDM15 培养基稀释培养物（培养物体积：待添加的 HEK293 CDM15 培养基体积=1:1）；

2.6.2.3.According to the process of viral infection, continue to culture and harvest after adding the virus.

根据病毒培养工艺，接种病毒，并继续培养病毒至收获。

Notices 注意事项

1. After hydrating HEK293 CDM15 medium, 2 ~ 4 mM Glutamine should be added (according to actual consumption).

   HEK293 CDM15 配制成液体时，根据消耗，需补加2 ~ 4mM谷氨酰胺；

2. Cells should be passaged in time. If the cell density is very high, which will result in the decline of cell state, the increase of dead cells, cell debris and cell clumping.

   细胞应及时传代。如果细胞密度很高，会导致细胞状态下降，死亡细胞增多，细胞碎片增多，细胞聚集；

3. Liquid cell culture medium should not be exposed to light or heat for a long time and stored in 2 ~ 8°C away from light.

   液体细胞培养基不宜长时间光照或热照，应避光保存在2 ~ 8°C。