V02

细胞上清外泌体提取试剂盒(沉淀法)

货号：EX0016
规格：50T(10mL)/250T(50mL)
保存：2-8℃保存，有效期 1 年。

产品介绍：

外泌体(Exosome)是膜包裹的细胞外囊泡(Extracellular vesicles, EVs)，直径约为 40-160nm，具有脂质双分子层结构，天然存在于血液、尿液、脑脊液，以及体外培养细胞的上清液中，几乎所有类型的细胞都可以产生并释放外泌体。外泌体可以被附近或远距离的细胞识别和融合，是细胞间进行相互调控的重要媒介，参与了癌症、神经退行性病变和炎症性疾病等多种疾病的发病过 程，影响细胞多方面的功能。

细胞上清外泌体提取试剂盒(沉淀法) (Exosome Isolation Kit, Precipitation Method, For Cell Culture Media)，也称为外泌体抽提试剂盒，是一种高效、便捷的用于细胞培养液上清中外泌体或其它细胞外囊泡提取的试剂盒，采用多聚物沉淀法，设备要求低、操作方便、提取时间短、样品起始量低、提取效率高、外泌体形态比较完整。使用本试剂盒提取的外泌体可用于蛋白分析、核酸分析、Western blot、PCR、RNA 提取、高通量测序、外泌体粒径和浓度分析即纳米颗粒示踪分析(Nanoparticle tracking analysis, NTA)、电镜分析、细胞共培养等实验。

操作步骤：*(仅供参考)*

一、 样品的准备

1. 在适当的条件下培养所需细胞，当细胞密度达 70%-80%时(处于对数生长期)，加入不含外泌体的血清培养液或适当的的无血清培养液，继续培养 12-24 小时，待细胞密度达 90%-100%时，收集细胞上清。

注 1：由于血清中含有非常多的外泌体，为了避免污染，此时需要加入不含外泌体的血清。不含外泌体的血清可以通过超速离心法获得，也可以直接使用无外泌体的血清。也可以根据具体的实验条件，使用不含血清的培养液进行培养，某些细胞可无血清正常生长约 12 个小时，或者增加不含细胞的培养液组作为阴性对照。

注 2：细胞上清中外泌体的量随细胞类型、细胞状态和细胞数量的差别而有一定的差异，须根据实验需求决定样品的起始量。

注 3：细胞凋亡、死亡过程中会释放大量囊泡，这些囊泡在外泌体的提取纯化过程中会污染活细胞产生的外泌体，请确保细胞状态良好，凋亡或死亡细胞占比尽量不超过 5%。

2. 将收集的细胞培养液在 4ºC，500×g 离心 5 分钟，轻轻缓慢地吸取上清液至一新的离心管中；再将上清液在 4ºC，10,000-16,500 ×g 离心 30 分钟，轻轻缓慢地吸取上清液至一新的离心管中。

3. 用 0.22μm 的针头滤器过滤上清液，进一步去掉较大的细胞囊泡和凋亡小体等杂质，将过滤后的上清液转移至新的离心管中。

4. （*可选*）使用 10-100kDa 之间的超滤管对上清液进行浓缩。将浓缩后的上清液从超滤管的死体积收集器中取出，用于后续外泌体提取。

注：一些细胞(干细胞、神经细胞)分泌的外泌体较少，可以将细胞上清液体积浓缩 10 倍左右再进行后续的外泌体提取；而对于外泌体分泌量多的肿瘤细胞等，可以不进行浓缩或将细胞上清液体积浓缩 2-5 倍左右在进行后续的外泌体提取。具体浓缩倍数可根据实际情况进行调整。

二、 外泌体提取

1. 每 1ml 步骤 1 中准备好的上清液样品中加入 190μl 细胞上清外泌体提取试剂，吹打混匀后置于 4ºC，静置 4 小时或者过夜。

注：外泌体提取试剂非常粘稠，需缓慢吸取，缓慢加入，并确保外泌体提取试剂与细胞上清液充分混匀。如果细胞分泌的外泌体较少，则可以通过增加静置的时间以提高外泌体得率。

2. 10,000×g 在 4ºC 离心 30 分钟，用 1ml 吸头小心吸除上清液，尽可能吸净上清液但不要触碰沉淀，收集沉淀，即为外泌体。

注：细胞培养液样品中外泌体一般含量较少，此时可能肉眼观察不到沉淀，如果离心时使用角转子，注意标记离心管摆放方向，并在底部位置画圈标识。

3. 离心获得的外泌体沉淀可用适量的 PBS 或者生理盐水重悬，一般 10ml 细胞培养液的起始量使用 0.1-1ml

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重悬液进行重悬；也可直接将沉淀用于后续实验。

注：外泌体可在 4ºC 保存 1 周，或在-20ºC及更低温度长期保存。

4. （*可选*）某些样品中的非外泌体杂质较多，导致沉淀比较多，此时可通过重悬并短暂离心去除杂质。将沉淀用适量的 PBS 重悬， 然后 12,000×g 在 4ºC 离心 2 分钟，取上清。若沉淀较多，可将上清多次 12,000×g 在 4ºC 离心 2 分钟，直至无明显沉淀。

5. 注 1：沉淀可能较难重悬，需反复多次吹打。

6. 注 2：需根据后续的纯化方法选择合适的重悬液。

三. **外泌体纯化(*可选*)**

1. 获得的外泌体可通过外泌体纯化柱或亲和层析法等方法进一步进行纯化。

2. 若需要获得无菌的外泌体，则可使用 0.22μm 的针头滤器进行过滤。为减少损失，请先将滤器用 PBS 进行润洗。若提取的外泌 体暂时不使用，可进行分装后于-80ºC 保存。

注意事项：

1. 本产品较为粘稠，使用时需完全混合均匀后再吸取，并保证吸取体积准确。
2. 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
3. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

相关产品：

K007602P	*Anti-CD63 Polyclonal Antibody*
K001361M	*Anti-Hsp70 Monoclonal Antibody*
K106583P	*Anti-HSPA1A Polyclonal Antibody*
K000385P	*Anti-CD81 Polyclonal Antibody*
K114124P	*Anti-TSG101 Polyclonal Antibody*
EX0010	*血清血浆外泌体提取试剂盒*
EX0011	*细胞上清外泌体提取试剂盒*
EX0012	*外泌体提取试剂盒（尿液）*
EX0013	*外泌体提取试剂盒（乳液）*
EX0015	*外泌体定量检测试剂盒*

V02

Exosome Isolation Kit, Precipitation Method, For Cell Culture Media

Cat: EX0016
Size: 50T(10mL)/250T(50mL)
Storage: 2-8℃, valid for 1 year.

Introduction

Exosomes are membrane-coated Extracellular vesicles (EVs), approximately 40-160nm in diameter, with a lipid bilayer structure, naturally occurring in blood, urine, cerebrospinal fluid, and the supernatant of cultured cells. Exosomes are produced and released by almost all types of cells. Exosomes, which can be recognized and fused by nearby or distant cells, are an important medium for mutual regulation between cells. They are involved in the pathogenesis of many diseases, such as cancer, neurodegeneration and inflammatory diseases, and affect various functions of cells.

Exosome Isolation Kit, Precipitation Method, For Cell Culture Media, also known as exosome extraction kit, It is an efficient and convenient kit for the extraction of exosomes or other extracellular vesicles in the supernatant of cell culture medium. The polymeric precipitation method is adopted, which has low equipment requirements, convenient operation, short extraction time, low sample starting amount, high extraction efficiency, and relatively complete exosomes morphology. Exosomes extracted using this kit can be used for protein analysis, nucleic acid analysis, Western blot, PCR, RNA extraction, high-throughput sequencing, exosome size and concentration analysis (Nanoparticle tracking analysis,NTA),electron microscope analysis, cell co-culture and other experiments.

Protocol(*for reference only*)

First. Sample Preparation

1. Culture the required cells under appropriate conditions, when the cell density reaches 70%-80% (in the logarithmic growth phase), add serum culture medium without exosome or appropriate serum-free culture medium, continue to culture for 12-24 hours, and collect the cell supernatant when the cell density reaches 90%-100%.

Note 1: Since the serum contains a large number of exosomes, in order to avoid contamination, it is necessary to add serum without exosomes at this time. Exosom-free serum can be obtained by hypercentrifugation, or exosom-free serum can be used directly. It can also be cultured using serum-free culture medium according to specific experimental conditions, and some cells can grow normally without serum for about 12 hours, or the cell-free culture medium group can be added as a negative control.

Note 2: The amount of exosomes in the cell supernatant varies with the cell type, cell state and number of cells, and the starting amount of the sample must be determined according to the experimental needs.

Note 3: A large number of vesicles are released during the process of apoptosis and death, and these vesicles will contaminate the exosomes produced by living cells during the extraction and purification of exosomes. Please ensure that the cells are in good condition and the proportion of apoptotic or dead cells does not exceed 5%.

2. Centrifuge the collected cell culture medium at 500×g at 4ºC for 5 minutes, and gently and slowly absorb the supernatant into a new centrifuge tube; Centrifuge the supernatant at 4ºC, 10,000-16,500 ×g, for 30 minutes, and gently and slowly draw the supernatant into a new centrifuge tube.
3. Use a 0.22μm needle filter to filter the supernatant, further remove impurities such as large cell vesicles and apoptotic bodies, and transfer the filtered supernatant to a new centrifuge tube.
4. (*Optional*) Use an ultrafiltration tube between 10-100kDa to concentrate the supernatant. The concentrated supernatant was removed from the dead volume collector of the ultrafiltration tube for subsequent exosome extraction.

Note: Some cells (stem cells, nerve cells) secrete less exosomes, so the supernatant liquid can be concentrated about 10 times before subsequent exosomes extraction; For tumor cells with a large amount of exosomes secreted, the concentration can be avoided or the cell supernatant liquid can be concentrated by about 2-5 times for subsequent exosome extraction. The specific enrichment ratio can be adjusted according to the actual situation.

Second, Exosome Extraction

1. Every 1ml of supernatant sample prepared in Step 1, 190μl of extractor for extractor of cell supernatant was added to the sample. After blowing and mixing, it was placed at 4ºC and left for 4 hours or overnight.

Note: Exosome extraction reagent is very viscous, it should be slowly absorbed, slowly added, and ensure that the

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exosome extraction reagent and the cell supernatant are fully mixed. If the cell secretes fewer exosomes, the exosome yield can be increased by increasing the resting time.

2.10,000×g centrifuge at 4ºC for 30 minutes, carefully remove the supernatant with a 1ml suction head, and absorb the supernatant as much as possible without touching the precipitation, and collect the precipitation, which is the exosome.

Note: The content of exosomes in the cell culture fluid sample is generally small, and the precipitation may not be visible to the naked eye at this time. If the angular rotor is used during centrifugation, note the direction of the centrifuge tube and mark the bottom position with a circle.

3. Exosome precipitates obtained by centrifugation can be re-suspended with appropriate amount of PBS or normal saline. Generally, the initial amount of 10ml cell culture medium is re-suspended with 0.1-1ml of re-suspension. Precipitation can also be directly used for subsequent experiments.

Note: Exosomes can be stored for 1 week at 4ºC, or long-term at -20 ℃ and lower.

4. (*Optional*) There are more non-exosome impurities in some samples, resulting in more precipitation, and the impurities can be removed by re-suspension and brief centrifugation. The precipitate was re-suspended with appropriate amount of PBS, then centrifuged at 12,000×g at 4ºC for 2 min, and the supernatant was taken. If there is too much precipitation, the supernatant can be centrifuged at 4ºC for more than 12,000×g for 2 minutes until there is no obvious precipitation.

Note 1: Precipitation may be difficult to re-hang, need to be blown repeatedly.

Note 2: Appropriate suspensions should be selected according to subsequent purification methods.

Third. Exosome Purification (*optional*)

1. The obtained exosomes can be further purified by exosome purification column or affinity chromatography.
2. If sterile exosomes need to be obtained, a 0.22μm needle filter can be used for filtration. In order to reduce the damage, please wash the filter with PBS first. If the extracted exosomes are not used for the time being, they can be stored at -80ºC after packaging.

Note

1. This product is sticky, it should be completely mixed and absorbed before use, and ensure that the absorption volume is accurate.
2. This product is only used for scientific research by professionals, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, and shall not be stored in ordinary homes.
3. For your safety and health, please wear a lab coat and disposable gloves.

Related products

K007602P	*Anti-CD63 Polyclonal Antibody*
K001361M	*Anti-Hsp70 Monoclonal Antibody*
K106583P	*Anti-HSPA1A Polyclonal Antibody*
K000385P	*Anti-CD81 Polyclonal Antibody*
K114124P	*Anti-TSG101 Polyclonal Antibody*
EX0010	*Exosome Isolation Kit, EPF Method，For Blood Sample*
EX0011	*Exosome Isolation Kit, EPF Method，For Cell Culture Media*
EX0012	*Exosome Isolation Kit, EPF Method，For Urine Sample*
EX0013	*Exosome Isolation Kit, EPF Method，For Emulsion Sample*
EX0015	*Exosome Quantitative Detection Kit*