Wayen Exosome Isolation Kit

User Manual

Cat# EIQ3-04001（Cell Culture Media） Version 2017-01

Description (For research only)

EIQ3-04001 kit is used to isolate exosomes between 30 and 200 nm diameter from cell culture media. By adding appropriate amount of reagent to the cell culture media, and incubating the mixture over 12 h, the exosomes can be collected with centrifugation.

Advantages

- ✓ Quantity: Higher yield
- ✓ Easy: No ultracentrifugation

Contents

EIQ3-04001 kit contains one reagent.

Storage

The kits are shipped at room temperature and should be properly stored after receipt. Properly stored kits are stable for 1 year from the date received.

Components	Storage	Volume
Reagent	4 ℃	50 mL

Experiment Protocol of EIQ3-04001（Cell Culture Media）

1. Prepare Sample

1.1 Take the cell culture media from storage and keep it on ice. If starting with frozen sample, thaw the sample completely in a 25 ℃ water bath and then place it on ice.

1.2 Centrifuge the cell culture media at 3,000 ×g for 15 minutes at 4 ℃.

1.3 Transfer the supernatant to a new tube and place it on ice until ready to perform the isolation.

2. Isolate Exosomes (Balance the Reagent to room temperature before use and the starting volume of cell culture media is recommended to be 10 mL. The protocol below is shown with 10 mL cell culture media.)

2.1. Take out 10 mL pre-treated cell culture media, add 5 mL Reagent into it and invert the mixture up and down until obtain a homogenous mixture.
※**Note: Cell culture media : Reagent =2:1 (volume)**

2.2 Incubate the mixture at 4 ℃ overnight (12-16 h).

2.3 After incubation, centrifuge at 3,000 ×g for 60 minutes at 4 ℃.

2.4 Take out 1 mL supernatant into a 1.5 mL tube firstly and remove the residual supernatant.

2.5 Resuspend the pellet completely with 1 mL supernatant above. Transfer the mixture to a new 1.5 mL tube.

2.6 Centrifuge the re-suspension at 10,000 ×g for 10 minutes at 4 ℃. Remove the supernatant without disturbing the precipitated pellet.

2.7 Resuspend the pellet with 50-200 μL 1 ×PBS, and mix it well by vortexing or pipetting up and down until obtain a homogenous mixture.

2.8 Centrifuge the sample again at 10,000 ×g for 5 minutes at 4 ℃. Transfer the supernatant to a new tube.

2.9 The supernatant contains exosomes. The exosomes can be used for downstream analysis immediately or aliquoted and stored at – 80 ℃ till next experiment.

Notice

This kit is for research use only, not for clinical diagnostic purpose.
1 ×PBS is not supplied and should be prepared by user.
We recommend that exosomes used for electron microscopy, NTA analysis and proteomics studies should be filtered by 0.22 μM filtration.
For more detail information, please visit our official website: www.wayenbio.com

华盈生物外泌体提取试剂盒

使用说明书

货号 EIQ3-04001（细胞培养上清）版本 2017-01

产品描述(只应用于科研)

EIQ3-04001 试剂盒能从细胞培养上清样本中分离纯化出粒径范围在 30 – 200 nm 的外泌体。在细胞培养上清样本中加入适量的提取试剂，经过夜孵育后，即可以通过普通离心收集外泌体。

技术优势

✓ 高量: 相对于其它方法，能够提取更高产量的外泌体

✓ 便利: 操作简单，无需超速离心

试剂组成

试剂盒仅包含一种试剂。

储存条件

室温条件下运输，4 ℃ 储存，保质期 1 年。

成份	储存条件	试剂量
试剂	4 ℃	50 mL

基本信息

试剂盒仅适用于细胞培养上清的外泌体提取，每次反应可处理 10 mL 样本。提取其它类型样本的外泌体，建议使用其他专业型试剂盒。

操作步骤:（细胞培养上清）

1. 样本准备

1.1 细胞培养上清样本需冰上放置，如初始样本为冻存样本，需在 25 ℃ 水浴中解冻，至其完全融化后置于冰上；

1.2 取适量细胞培养上清，4 ℃， 3,000 ×g，离心 15 min；

1.3 转移上清至新的离心管中，置于冰上。

2. 外泌体提取（注：试剂使用前需平衡至室温，下面以 10 mL 细胞培养上清样本的提取为例。）

2.1 取离心过的细胞培养上清样本 10 mL，加入 5 mL 提取试剂，上下翻转使其混合均匀；

注：细胞培养上清量：试剂量 = 2:1（体积比）

2.2 将上述混合液，4 ℃，静置，孵育过夜（12-16 h）；

2.3 孵育结束后，将上述混合液，4 ℃，3,000 ×g，离心 60 min；

2.4 离心后，先取出 1 mL 上清液置于 1.5 mL EP 管中，然后弃去其他残留上清液；

2.5 用预先取出的 1 mL 上清液反复吹打管底以及侧壁，使沉淀充分重悬，并将重悬液转移至新的 1.5 mL EP 管中；

2.6 重悬液 4 ℃，10,000 ×g，离心 10 min，弃上清；

2.7 所得沉淀用 50-200 μL 1 ×灭菌 PBS 重悬，并反复吹打均匀；

2.8 将 2.7 所得重悬液 4 ℃，10,000 ×g，再次离心 5 min，取上清；

2.9 所得上清即为细胞培养上清外泌体的 PBS 重悬液，该上清可直接进行后续的分析或者实验，也可以分装后保存于-80 ℃，以便下游分析使用。

注意事项：

本试剂盒只应用于科学研究，不可应用于临床诊断。

1 ×PBS 不提供，需客户自己准备。

建议外泌体在进行电镜检测、NTA 分析以及蛋白组学研究前使用 0.22 μ m 小型过滤器进行过滤。

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