Wayen Exosome Isolation Kit

User Manual

Cat# EIQ3-03001（Urine） Version 2017-01

Description(For research only)

EIQ3-03001 kit is used to isolate exosomes between 30 and 200 nm diameter from urine. By adding appropriate amount of reagents (A and B) to urine sample, and incubating the mixture over 12 h, the exosomes can be collected with centrifugation.

Advantages

- ✓ Quantity: Higher yield (versus other kits or methods)
- ✓ Easy: No ultracentrifugation

Contents

EIQ3-03001 kit contains Reagent A and B.

Storage

Reagent A is shipped at room temperature and Reagent B is shipped at 4 °C. The kits should be stored properly after receipt. Properly stored kits are stable for 1 year from the date received.

Components	Storage	Volume
Reagent A	Room Temperature	37.5 mL
Reagent B	-20 °C	3.35 mL

Experiment Protocol of EIQ3-03001（Urine）

1. Prepare Urine Sample

1.1 Take the urine sample from storage and keep it on ice. If starting with frozen sample, thaw the sample completely in a 37 °C water bath and then place it on ice.

1.2 Centrifuge the urine sample at 3,000 × g for 15 minutes at 4 °C.

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 A to room temperature before use and the starting volume of urine is recommended to be 20 mL. The protocol below is shown with 20 mL urine.)

2.1 Take out 20 mL pre-treated urine, add 7.5 mL Reagent A, mix the mixture well by inverting it up and down until obtain a homogenous mixture.
Note: For every 10 mL of urine, 3.75 mL Reagent A should be added. When the start urine volume is changed, the volume of the reagent A should also be changed.

2.2 Add 670 μL Reagent B to the mixture above and invert the tube up and down to obtain a homogenous mixture.
Note: For every 10 mL of urine, 335 μL Reagent B should be added. When the start urine volume is changed, the volume of the reagent B should also be changed.

2.3 Incubate the mixture at 4 °C overnight (12-16 h).

2.4 After incubation, centrifuge at 3,000 × g for 60 minutes at 4 °C.

2.5 Take out 1 ml supernatant into a 1.5 mL tube firstly and then remove the residual supernatant.

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

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

2.8 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.

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

2.10 The supernatant contains exosomes. The exosomes can be used for downstream analysis immediately or aliquoted and stored at -80 °C till next experiment.

Notice

This kit is for research use only, not for clinical diagnostic purpose.
1 × sterile 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 EIQ3-03001（尿液）版本 2017-01

产品描述(只应用于科研)

EIQ3-03001 试剂盒能从尿液样本中分离纯化出粒径范围在 30 – 200 nm 的外泌体。在尿液样本中加入适量的提取试剂 A 以及提取试剂 B，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；
- ✓ 方便: 操作简单，无需超速离心

试剂组成

试剂盒包含试剂 A 和试剂 B 两种试剂。

储存条件

试剂 A 在室温条件下运输，试剂 B 在 4 °C 条件下运输；试剂收到后应按照要求储存，保质期 1 年。

成份	储存条件	试剂量
试剂 A	室温	37.5 mL
试剂 B	-20 °C	3.35 mL

基本信息

试剂盒仅适用于尿液样本的外泌体提取工作，每次反应可处理 20 mL 尿液。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:（尿液）

1. 尿液样本准备

1.1 尿液样本需冰上放置，如初始尿液样本为冻存样本，需在 37 °C 水浴中解冻，至其完全融化后置于冰上；

1.2 尿液样本，4 °C，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：提取试剂 A 使用前需平衡至室温，建议尿液样本的起始量为 20 mL，以下反应以 20 mL 尿液外泌体的提取为例。）

2.1 取离心过的尿液样本 20 mL，加入 7.5 mL 提取试剂 A，上下翻转使其混合均匀；

注：每 10 mL 尿液需加入 3.75 mL 提取试剂 A，更多的初始尿液量需按照相应的比例进行调整

2.2 向上述混合液中加入 670 μL 提取试剂 B，上下翻转使其充分均匀；

注：每 10 mL 尿液需加入 335 μL 提取试剂 B，更多的初始尿液量需按照相应的比例进行调整

2.3 将上述混合液，静置，4 °C，孵育过夜（12-16 h）；

2.4 孵育结束后，4 °C，3,000 × g，离心 60 min；

2.5 离心后先取出 1 mL 上清液置于 1.5 mL EP 管中，然后完全去除其他残留上清液；

2.6 用上述取出的残留液反复吹打管底，使沉淀充分重悬，并将重悬液转移至 1.5 mL EP 管中；

2.7 所得重悬液 4 °C，10,000 × g，离心 10 min，弃上清；

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

2.9 将上述重悬液 4 °C，10,000 × g，离心 5 min，取上清，转移至新的 1.5 mL 的 EP 管中；

2.10 所得上清即为尿液外泌体的 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 °C，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 ×灭菌 PBS 试剂盒不提供，用户需自己准备。

建议外泌体进行电镜检测、NTA 分析以及蛋白组学等研究前，使用 0.22 μm 小型过滤器进行过滤。

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