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A fast, easy, cost-free method to remove excess dye or drug from exosome solution V.1

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We use this protocol and it's working

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Disclaimer

This protocol has been submitted to PLOS ONE and it is under the revision process

Abstract

This protocol details a cost-free method to remove excess dye or drug from exosome solution.

Attachments

PDF

pny6cbzb7.pdf

226KB

Guidelines

Tips:

- Cells from step 5 should have signal detectable signal under flow cytometry and/or fluorescent microscopy assay (sponge cells). In case they do not, it is advised to increase the concentration of the dye to ensure that all isolated particles are sufficiently stained.
- Cells from step 9 should have no signal (test cells). If they do, it is advised to optimize the staining conditions. For example, a reduction in the initial amount of dye can be considered. Alternatively, an increase in the number of parental cells used per washing step can also be explored with a caveat that this might reduce the number of exosomes remaining.

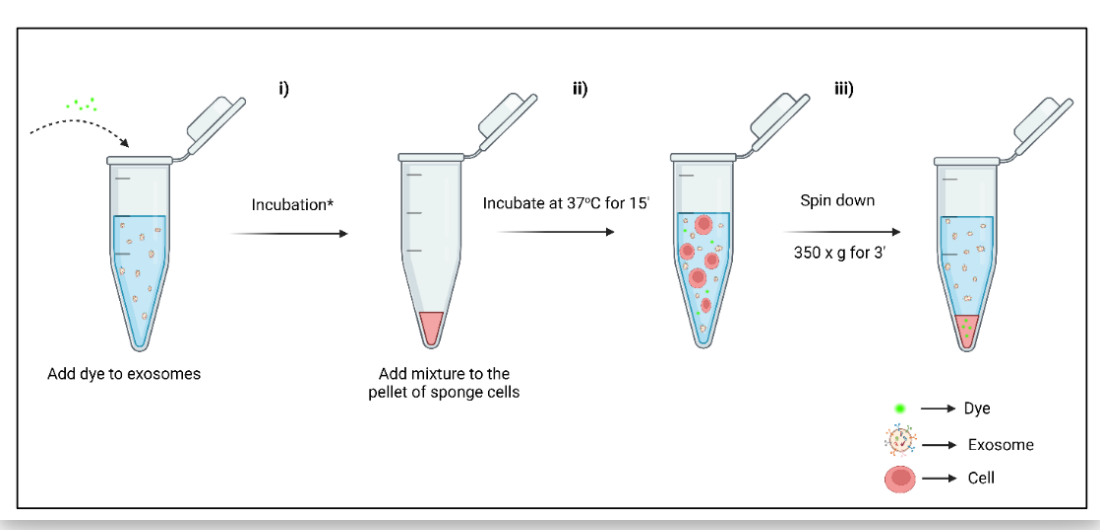

Fig. 1. Overview of the protocol to remove the excess dye from the solution containing the stained exosomes. i) Exosomes incubated with the respective dye/drug. The time and the conditions of this incubation are dependent on
the exosome type and the recommendation of the dye/drug supplier. In our case, KG1a-derived exosomes were stained with 2μM of DiO (or 6μM of DNR) for 1 hour at 37 o C shaking at 350 rpm [1]. ii) The mixture from step i is added to a pellet of parental-sponge cells (here KG1a cells) and incubated for 15 minutes at 37o C with shaking at 350 rpm. iii) The mixture of exosomes with sponge cells were centrifuged for 3 minutes at 350 x g and the resulting
supernatant containing the stained exosomes, without excess dye/drug, is ready for downstream applications. Figure created by BioRender.com.

Materials

Centrifuge (Eppendorf 5415R)

Protocol Steps

36m

1 Collect 0.5 x 10^6 cells (preferably parental cells used for exosome isolation)* and wash them with
phosphate buffered saline (PBS) or alternative buffer.

Note

*Since exosomes derived from their parental cells usually share the same ligands, the likelihood of their ligands interacting is lower.

2 Pellet down the cells (typically 350 x g, Room temperature, 00:03:00).

3m

3 Add the mixture containing the stained exosomes** with the excess dye/drug to the cell pellet.

Note

** The proposed cleaning protocol has been tested to a range from 100,000 to 1,000,000 exosomes.

4 Resuspend by gently pipetting the cells and leave the mixture shaking at
350 rpm, 37°C, 00:15:00

15m

5 Spin down the mixture so the cells form a pellet using the same parameters as in step 2.

6 Transfer the supernatant into a new clean tube. This supernatant should now contain the stained or loaded exosomes without any excess dye in the buffer solution used.

Note

To optimize the protocol, whenever new types of exosomes/dye are being used, it is advisable to continue with the following steps:

7 Repeat steps 2 through 5:

18m

- Pellet down the cells (typically 350 x g, Room temperature, 00:03:00).
- Add the mixture containing the stained exosomes** with the excess dye/drug to the cell pellet.
- Resuspend by gently pipetting the cells and leave the mixture shaking at 350 rpm, 37°C, 00:15:00
- Spin down the mixture so the cells form a pellet using the same parameters as in step 2.

8 Collect the supernatant which contains the stained exosomes without the excess dye.

9 Resuspend the pellet in PBS buffer (or alternative) and check whether the cells (test cells) were stained. This confirmation step can be verified using fluorescent microscopy or flow cytometry for fluorescent drugs or dyes.

Protocol references

1. **Isaioglou, I., et al.**, CD34(+) HSPCs-derived exosomes contain dynamic cargo and promote their migration through functional binding with the homing receptor E-selectin. Front Cell Dev Biol, 2023. 11: p. 1149912.