Document Number:	**SASoM/METHOD/045.v4**
Title:	**Exosome Red Fluorescent Labelling**
Version:	**v4**
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Valid to:	09/08/2021

SOP History		
Number	Date	Reason for Change
v1	02/08/2013	Original
v2	10/08/2015	Update
v3	10/08/2017	Update and revised author
V4	10/08/2019	Update

1.0 Purpose –
This SOP describes the current procedure for **Exosome Red Fluorescent Labelling** for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in **Exosome Red Fluorescent Labelling** work.

3.0 Responsibilities –
All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

Sigma-Aldrich PKH26 red fluorescent cell linker kits for general cell membrane labelling (catalogue numbers MINI26 and PKH26GL)

N.B. All procedures should be carried out in the fume hood to avoid contamination of samples

1. Extract exosomes from sample as per steps 1 – 9 of the 'Exosome isolation using centrifugation' SOP.

2. Ensure removal of all the final supernatant after step 8 (100,000 x g spin for 120 minutes at 4°C). (The presence of physiological salts causes the dye to form micelles and substantially reduces staining efficacy.)

3. Resuspend exosomes in 1ml Diluent C (catalogue number G8278). Use the pipette tip to disturb the pellet (taking care to avoid the formation of bubbles), then ensure complete dispersion by pipetting.

4. (Steps 4 – 8, inclusive, must occur in a polypropylene tube to minimise uptake of dye by centrifuge/conical tube. If not polypropylene centrifuge tube is available transfer the resuspended sample from the end of step 3 to a polypropylene tube, then return the sample at the end of step 8 to an appropriate centrifuge tube.)

5. Avoid exposure to light source to preserve the fluorescent dye in subsequent steps.

6. Add 2 µl PKH26 red fluorescent cell linker and immediately mix the sample by pipetting.

7. Allow labelling to occur for 5 minutes in a dark room.

8. Add 3 ml 1% BSA and incubate for 1 minute (or other suitable protein solution, to stop membrane staining by allowing binding of excess dye).

9. Centrifuge the sample at 100,000 x g (Beckman Coulter Optima L-100 XP Ultracentrifuge; SW 55 rotor; 34,000 RPM) for 120 minutes at 4°C to pellet the labelled exosomes.

10. Resuspend the exosomes then wash them by three further centrifuges (100,000 x g, 120 minutes at 4°C).

11. Resuspend in relevant exosome-free medium/buffer (now exosome membranes have been labelled there is no need to avoid solutions containing physiological salts as there is no free fluorescent dye).

12. (The supernatant at this point can then be used to quantify the exosome content of the sample.)

13. (The exosomes can now be stored at -20^{o}C until needed for further use.)

5.0 Personal protection -
A Howie laboratory coat and lab gloves must be worn at all times.

6.0 Spillages -
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training -
All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments –COSHH/004 and RA/BIOL/004

8.2 SOP SASoM/METHOD/042
Exosome Isolation Using Antibody Coated Beads

8.3 SOP SASoM/METHOD/043
Exosome Isolation Using Centrifugation

8.4 SOP SASoM/METHOD/044
Exosome Quantification Using NanoSight Tracking Analysis

8.4 SOP SASoM/METHOD/044
Exosome Red Fluorescent Labelling

9.0 Approval and sign off –

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