Document Number: **SASoM/METHOD/042.v4**

Title: **Exosome Isolation Using Antibody Coated Beads**

Version: **v4**

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

1.0 Purpose –

This SOP describes the current procedure for **Exosome Isolation Using Antibody Coated Beads** 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 Isolation Using Antibody Coated Beads** 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 –

This technique uses antibodies to exosome surface proteins CD 9, CD 45 & CD 63. These are frequently used antibodies (e.g. these are common surface proteins for T cells), however antibodies to other surface proteins could equally be used.

Antibodies were obtained from Santa Cruz Biotech, catalogue numbers:

- Mouse anti-CD 9: SC 51575
- Mouse anti-CD 45: SC 53666
- Mouse anti-CD 63: SC 51662

Magnetic antibody coated beads from Invitrogen: Invitrogen Magnetic Dynabeads, concentration 4 x 10^8 beads/ml, coated with sheep anti-mouse IgG (catalogue number 110.31).

1. Pipette 40 µl of Invitrogen Magnetic Dynabeads into a 1.5 ml Eppendorf tube placed in a magnetic rack.

2. Wash twice:
   a. Add 1 ml of serum-free RPMI medium for 1 minute (the beads will adhere to the sides of the tube next to the magnetic rack), the free medium can then be removed without disturbing the beads.
   b. Repeat part a.

3. Resuspend the beads in 40 µl of serum-free RPMI medium.

4. Aliquot samples in 1.5 ml Eppendorf tubes:
   a. 10 µl of serum-free RPMI medium (negative control).
   b. 10 µl of anti-CD 9, anti-CD 45 & anti-CD 63).

5. Add 10 µl of beads/serum-free suspension to each tube.

6. Incubate together for 2 hours at 4°C on an agitator (during this time the sheep anti-mouse antibodies coating the magnetic beads will bind the free mouse anti-CD free antibodies).

7. Return Eppendorf's to a magnetic rack and wash three further times (to remove cytotoxic sodium azide):
   a. Add 1 ml of serum-free RPMI medium for 2 minute (the beads will adhere to the sides of the tube next to the magnetic rack), the free medium can then be removed without disturbing the beads.
   b. Carry this out two further times finishing by removing the 1 ml of serum-free RPMI medium to leave only the magnetic beads in each Eppendorf tube.

8. Resuspend the magnetic beads in 100 µl of serum-free RPMI medium.

9. Add to a concentrated exosome sample and incubate overnight at 4°C on a rotating wheel/agitator/rocker.

10. Return the Eppendorf tubes to the magnetic rack.

11. Wash three times with 1 ml of serum-free RPMI medium, for two minutes each time.

12. Remove supernatant to leave magnetic beads coated with exosomes.

Further steps depend on desired use of the exosomes.

For Western blot:

13. Add 20 µl of reducing sample buffer (to cleave the exosomes from the magnetic beads and break open the exosomes).

14. Heat sample at 80°C for 1 minute (to help further denature the protein).

15. Centrifuge the sample for 20 seconds, at room temperature, on a table top centrifuge (to pellet the magnetic beads).

16. Load the 20 µl on an appropriate Western gel.

For functional studies:

17. Resuspend the magnetic bead-exosome complex in serum-free RPMI medium and add to cell sample.
(N.B. For this it is important to have a serum-free RPMI medium magnetic bead only solution for negative control.)

To assess with Nanocyte Tracking Analysis:

18. Resuspend the magnetic bead-exosome complex in 200 µl 0.2 Molar glycine (pH 2.8 – this cleaves the exosomes from the beads but does not break open the membranes).

19. Neutralise the pH by adding 5 µl Tris HCl (pH 11) for 5 minutes at 37°C.

20. Centrifuge the sample for 20 seconds, at room temperature, on a table top centrifuge (to pellet the magnetic beads).

21. Use the supernatant for NTA.

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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