

Method Procedure

Document N	Imber: SASoM/METHOD/105.v2		
Title:	In-Cell Western with Li-COR Secondary Antibodies using Licor Odyssey Scanner.		
Version:	/2		
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Valid to:	11/11/2022	

SOP History		
Number	Date	Reason for Change
v1	01/11/2018	Qriginal
V2	11/11/2020	Update

#### 1.0 Purpose –

This SOP describes the current procedure for performing 'In-Cell' westerns using the Licor Odyssey scanner in Laboratory 248 at the St Andrews School of Medicine (SASoM).

#### 2.0 Scope -

This SOP applies all staff /students in the SASoM performing 'In-Cell' westerns using the Licor Odyssey scanner.

# 3.0 Responsibilities -

All staff / students involved in performing 'In-Cell' westerns using the Licor Odyssey scanner are responsible for ensuring that the methods are followed in accordance with this SQP.

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



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# 4.0 Procedure –

A) Solutions and Reagents:

- 10X Phosphate Buffered Saline (PBS): dissolve 10 PBS tablets in 100ml ddH2O (OXOID, CAT NO. BR0014G).
- Methanol, cooled at -20°C at least for 1 hour.
- 4% Formaldehyde, newly made up in PBS before use.
- Blocking Buffer: To prepare 25 ml, add 2.5 ml 10X PBS, 1.25 ml normal serum from the same species as the secondary antibody (eg. normal goat serum, normal donkey serum) and 21.25 ml dH2O and mix well. While stirring, add 75 µl Triton X-100 (100%).
- Antibody dilution buffer: To prepare 40ml, add 4ml 10×PBS to 36ml dH2O, mix. Add 0.4g BSA and mix well. While stirring, add 120µl Triton X-100(100%).

<u>Specimen Preparation:</u> NOTE: Cells are grown and treated in black-walled, clearbottom 96-well microplate so as to prevent cross-contamination of light from well-towell.

B) Fixation: Formaldehyde – Methanol fixation.

- Aspirate media after drug treatment.
- Rinse wells with 200µl/well PBS (only if plate was used for AlamarBlue assay).
- Aspirate PBS from the wells (only if plate was used for AlamarBlue assay).
- Cover cells with 100 µl of 4% formaldehyde in each well.
- Allow cells to fix for 15 minutes at room temperature.
- Aspirate fixative, rinse three times in 200 µl/well of PBS for 5 minutes each.
- Permeabilize with 100 μH of ice cold 100% Methanol for 10mins in -20°C freezer.
- Wash three times in 200µ//well of PBS for 5mins each.

<u>C) Immunostaining</u>, NOTE: All subsequent incubations should be carried out at room temperature, unless otherwise noted, in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

- Block specimen in 40µl/well of Blocking Buffer for 60 minutes.
- While blocking, dilute primary antibody at a desired concentration in Antibody dilution buffer.
- Aspirate blocking solution, apply diluted primary antibody with 40µl/well. NOTE: For double-labelling, prepare a cocktail of the primary antibodies at their appropriate dilution in Antibody Dilution Buffer.
- Seal the plate with parafilm and Incubate overnight at 4°C (in the dark).

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- Wash three times in PBS for 5 minutes each.
- Incubate specimen in 40ul/well of 1:800 of IRdye680 (for the normalizer) and 1:800 of IRdye800 (for the target) diluted in Antibody dilution buffer for one hour at room temperature in the dark. Note that the dilutions of secondary antibodies are significantly different from those used in western blots.

- Wash in PBS as in step 5.
- Wait to dry before scanning on the Licor Odyssey scanner (dry samples generate a stronger signal than a wet sample).

#### 5.0 Personal protection –

A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

# 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 lowel, disinfected with 70% ethanol and finally washed with detergent.

# 7.0 Training –

All staff should undergo training in this technique before performing the procedure.

#### 8.0 Related documents –

- 8.1 Risk assessments RA/GEN/034; RA/COSHH/013; RA/COSHH/007
- 8.2 SOP SASoM/EQUIP/037 Licor Odyssey Scanner.



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# 9.0 Approval and sign off –

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