**Document Number:** **SASoM/METHOD/029.v4**

**Title:** **Optimising a New Antibody on the AQUA using the HistoR AQUAntiplex Epithelial Tissue Compartmentalisation Kit**

**Version:** **V4**

**Author:** **In Hwa Um**

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|---|---|
| Effective from: | 01/02/2019 |
| Valid to: | 31/01/2021 |

| **SOP History** | | |
|---|---|---|
| Number | Date | Reason for Change |
| V1 | 01/02/2013 | Original |
| V2 | 01/02/2015 | Update |
| V3 | 01/02/2017 | Update |
| V4 | 01/02/2019 | Update |
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## 1.0 Purpose –

This SOP describes the current procedure for optimising a new antibody on the AQUA using the HistoRx AQUAntiplex epithelial tissue compartmentalisation kit in Laboratory 248 at the St Andrews School of Medicine (SASoM).

## 2.0 Scope –

This SOP applies to the staff in the SASoM involved with optimising a new antibody on the AQUA using the HistoRx AQUAntiplex epithelial tissue compartmentalisation kit.

## 3.0 Responsibilities –

All staff involved in optimising a new antibody on the AQUA using the HistoRx AQUAntiplex epithelial tissue compartmentalisation kit 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 –**

Preparation

Check the antibody quality using western blotting. If the band is not clear enough or not specific enough (multiple bands) in the western blot, the antibody is not good enough for IF optimisation.

Read the antibody's datasheet thoroughly.

Plan for three different dilutions according to the manufacturer's recommendation e.g. if 1:200 is recommended by the manufacturer, use 1:100, 1:200 and 1:400 dilutions. If the antibody is not recommended for the usage of IHC or IF, then try 1:50, 1:100 and 1:200 dilutions.

Retrieve the antibody optimisation slides from the freezer (-20$^{O}$C) and label the slide with the antibody information such as antibody name, supplier name, catalogue number and dilutions.

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|---|---|---|---|
| Label (Record Ab name, Supplier, Cat. No, Dilutions) | 1:50 | 1:100 | 1:200 |

Dewax and Rehydration

Dewax in xylene for 2 x 5 min.

Rehydration for 2 min at 99%, 99%, 80%, 50% alcohol and running tap water.

Antigen Retrieval

Required solution should be boiled in a pressure cooker before placing slides in hot solution and heating for a further 5 minutes

Tris-EDTA pH9.0 or Sodium Citrate pH6.0 - pressure cooker for 5 min.

Cool down for 20min.

Rinse in 0.05% PBS-Tween 20 for 5 min, in a coplin jar, on a rocker.

Blocking

Draw a line around the triplicate TMAs using the water repellent DAKO pen (Dako, S2002).

Cover each TMA with 50-70µl of blocking buffer 1 (tube A from the HistoRx AQUAntiplex epithelial tissue compartmentalisation kit) for 5 min.

Rinse in 0.05% PBS-T for 5 min.

Cover each TMA with 50-70µl of blocking buffer 2 (tube B) for 10 min.

Rinse in 0.05% PBS-T for 5 min.

Primary antibody

Dilute the antibody with antibody cocktail (tube C). * Make sure to use the kit with the species of the primary antibody as there are two different AQUAntiplex epithelial kits - Rabbit target and Mouse target.

Add the antibodies onto the TMAs and incubate them for 1 hour at room temperature.

Rinse in 0.05% PBS-T for 3 x 5 min.

Epithelial mask visualisation

Cover each TMA with 50-70µl of epithelial mask visualisation (tube D) and incubate in the dark for 1 hour at room temperature.

Rinse in 0.05% PBS-T for 3 x 5 min.

Target visualisation

Combine the target signal amplification diluent and the Cy5 Tyramide at 1:50 concentration (tube E and F) and incubate in the dark for 10 min at room temperature.

Rinse in 0.05% PBS-T 3 x 5 min.

Dehydrate the slide in 80% Ethanol for 1 min.

Air dry in the dark.

Counterstaining and coverslipping

Apply 45µl Prolong Gold anti-fade reagent with DAPI (Invitrogen, P36931), nuclear visualisation media, on the coverslip (22 x 40mm) and place the coverslip over the tissue (for the smaller coverslips (22 x 26mm), apply 30µl).

Let the mounted slide dry overnight in the dark.

After slides are completely dried, seal the coverslips with nail polish.

AQUAscanning and AQUAnalysis

Scan the slide according to SOP SASoM/EQUIP/025 - Image Acquisition using HistoRx ® PM-2000, AQUA.

Analyse the three different diluted antibody stained TMAs according to SOP SASoM/EQUIP/026 - Image Analysis using HistoRx ® PM-2000, AQUA.

Reporting the optimisation result

Make sure the negative control is meeting the standard for negative control AQUA scores, see SOP SASoM/EQUIP/053 – Using an in-house quality control slide for immunofluorescent staining.

Make sure the antibody is expressed in an appropriate compartment such as nucleus, cytoplasm or membrane.

Make sure background noise is not too strong in the least dilution. If the stroma area has strong cy5 expression, then go to 'troubleshooting' below.

Make a graph in Microsoft Excel using the three different dilutions. AQUA normalised data which is from the appropriate compartment, e.g. ER should be expressed in nuclei compartment, use the AQUA scores form 'ER in nuclear compartment AQUA_NORM' and decide the best dilution.

Report the antibody optimisation result. (See attached Appendix 1.) The report must include Antibody name, supplier, catalogue number, species, storage condition, antigen retrieval, dilution tried, incubation condition, optimal dilution and antibody localisation.)

Troubleshooting

Too strong background noise

Redo optimisation diluting antibody further. If you have tried 1:50, 1:100 and 1:200 then try to do 1:200, 1:400 and 1:800. Try this until there is a specific staining pattern.

If all the cores are stained strongly in the weakest dilution

Redo optimisation diluting antibody further. If you have tried 1:50, 1:100 and 1:200 then try to do 1:200, 1:400 and 1:800. Try this until there is a specific staining pattern.

No signal in the strongest dilution

Try to use different antigen retrieval such as Tris-EDTA buffer pH9.0 or pepsin digestion.

Try a stronger dilution of the antibody.

**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 towel, 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 COSHH 8 & 19

8.2 SOP SASoMU/METHOD/024
Dewaxing and Rehydration of Paraffin Embedded Sections

8.3 SOP SASoM/EQUIP/015
Use of the Pressure Cooker for Antigen Retrieval

8.4 SOP SASoM/EQUIP/025
Image Acquisition using HistoRx ® PM-2000, AQUA.

8.5 SOP SASoM/EQUIP/026
Image Analysis using HistoRx ® PM-2000, AQUA.

8.6 SOP SASoM/METHOD/053
Using an in-house quality control slide for immunofluorescent staining.

## Appendix 1

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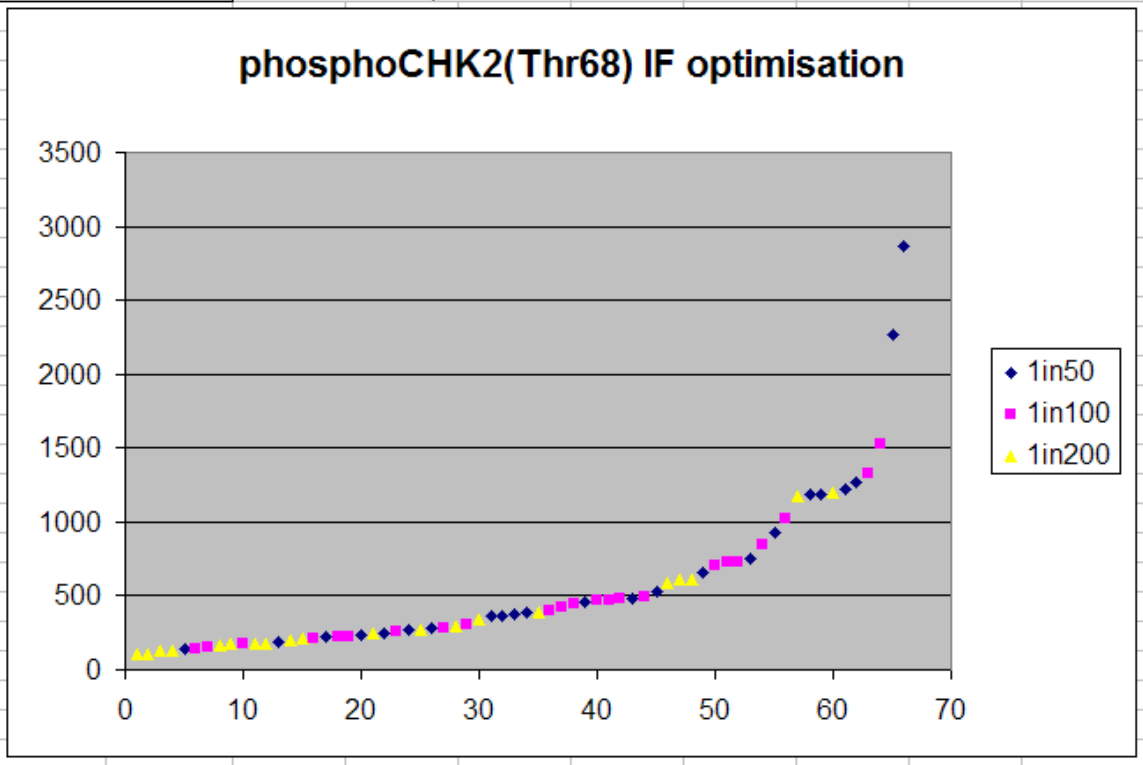
|---|---|
| **Antibody name** | **phospho CHK2 (Thr68)** |
| **Supplier** | Cell signaling |
| **Catalogue number** | 2197 |
| **Clone** | |
| **Species** | Rabbit |
| **Store at** | Freezer |
| **Antigen Retrieval** | NaCitrate pH6.0, pressure cooker 5mins |
| **Dilution** | 1 in 50,100 and 200 |
| **Incubation condition** | 1 hour at room temperature |

**Antibody optimisation report**

27-Jun-11

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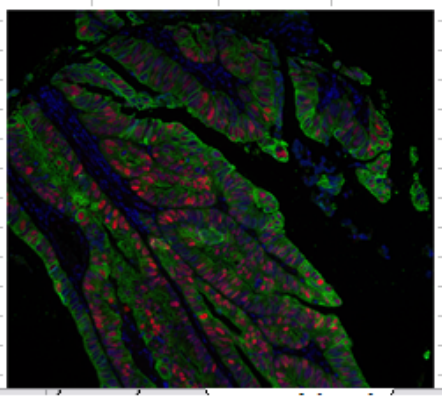
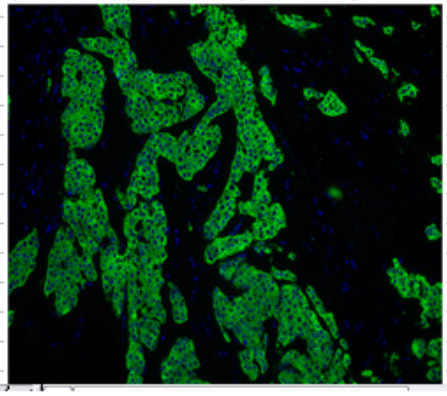
|---|---|
| **Antibody localisation** | Nuclei |
| **Optimal Dilution** | 1 in 50 |

AQUA score(Highest) 2871.323



AQUA score(lowest) 140.8338



## 9.0 Approval and sign off –

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|---|---|---|
| **Author:** | | |
| Name: | In Hwa Um | |
| Position: | Post Doc | |
| Signature: | | Date: |
| **Management Approval:** | | |
| Name: | Peter Mullen | |
| Position: | SOP Administrator | |
| Signature: | | Date: |
| **QA release by:** | | |
| Name: | Alex MacLellan | |
| Position: | QA Manager | |
| Signature: | | Date: |

# STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

| NAME | POSITION HELD | SIGNATURE | DATE |
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