Document Number: **SASoM/METHOD/086.v3**

Title: **MCM2 and Rabbit pan cytokeratin Immunofluorescence using Dako Link48**

Version: **v3**

Author: **In Hwa Um**

Effective from:	06/03/19
Valid to:	05/03/21

SOP History		
Number	Date	Reason for Change
v1		Original
v2	06/03/2017	A few steps added (4.15, 4.16)
V3	06/03/2019	Biennial Update

1.0 Purpose –

This SOP describes the current procedure for staining cytology samples and formalin fixed paraffin embedded tissue sections with MCM2 (Cytosystem) and Rabbit pan cytokeratin antibodies using Dako Link 48 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 MCM2 + rabbit pan cytokeratin immunofluorescence using Dako Link48.

3.0 Responsibilities –

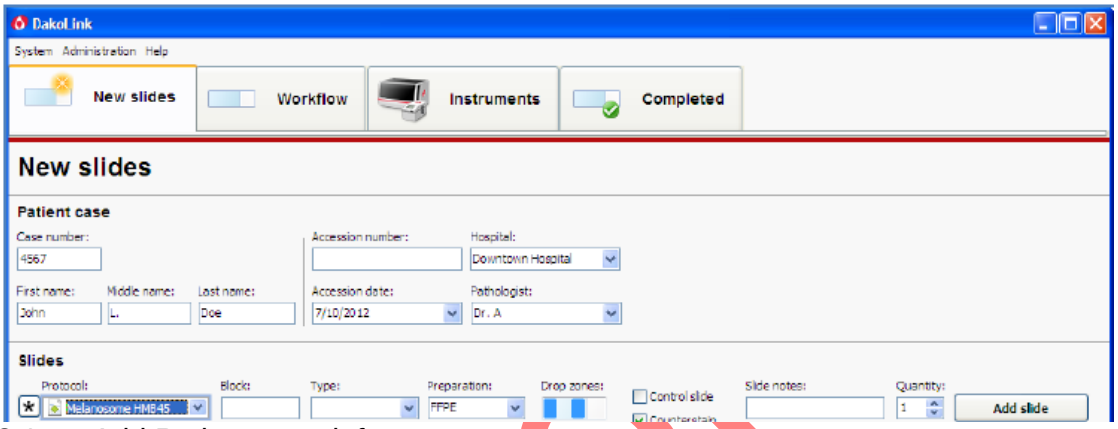
All staff involved in IF using DAKO Link48 are responsible for ensuring that the methods are followed in accordance with this SOP. All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

MCM2+Rabbit pan-cytokeratin immunofluroscence in Dako Link48

1. Turn on Dako automation system UPS and computer.
2. Double-click the DakoLink icon on the computer desktop and log in.

3. **Insert slide information in New slides tab**



3.1 Add Patient case info
3.2 Protocol-choose primary antibody **'MCM2+RCK'**, which includes '**USTAN IF MPrimary RabbitCK**' visualization system.
3.3 Block-skip
3.4 Type-skip
3.5 Preparation-choose either FFPE or Cytology
3.6 Drop zones-choose the drop zone appropriately

3.7 Slide notes-if necessary
3.8 Quantity
3.9 Print and apply the slide labels
3.10 Click 'Case complete'
3.11 Stick the label on the slides

4. Put the slide(s) in non-metallic rack. (No dewaxing and rehydration steps required)

Manual antigen retrieval

4.1 Make up EDTA-Tris 1 L solution:

10mM Tris Base = 1.21g
1mM EDTA = 0.37g
Tween = 0.5ml
dH20 = 1L
Add the EDTA and Tris Base to a 1L flask and add the dH20 and mix on magnetic stirrer. Ensure that the pH is 9. Add theTween.

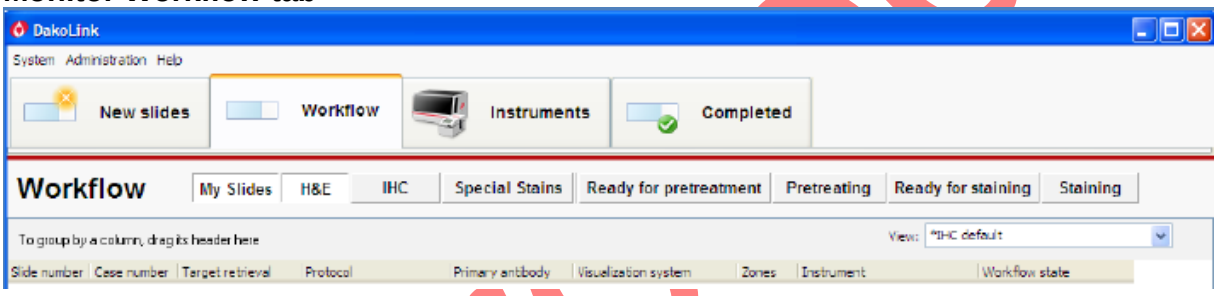
4.2 Pour the antigen retrieval solution into the microwave pressure cooker. Screw on the lid and put into the microwave on high power to heat up for 13mins.

4.3 Perform antigen retrieval on slides by putting slide rack into pre-heated pressure cooker. Screw on the lid and place the red weight over the vent. Put back into the microwave and heat on high for 5mins – there should be an audible hissing sound towards the end of the procedure.

4.3 Using heat resistant gloves and eye protection carefully lift the pressure cooker from within the microwave and place in sink. Place eye protection on. With the heat resistant gloves release the pressure by removing the weight from the top of the cooker. Stand well back immediately once the weight is removed. Do not stand over the pressure cooker during this step.

4.4 Allow the slides to cool down for 20mins. (Do not add cold tap water into the pressure cooker).

5. **Monitor Workflow tab**



5.1 My slides tab will show the slides you entered.

5.2 Determine and prepare required reagents using DAKO reagent bottles by registering them with Lot number and expiry date (5ml, 12ml and 25ml).

5.2.1 Endogenous enzyme block (Dako, #SM801) or equivalent to 3% H2O2 (Sigma, #H1009)

5.2.2 Dako protein block (Dako, #X0909)

5.2.3 Primary antibody- MCM2(Cytosystem, #03/0912, 1in500) + Rabbit pan-CK(Dako, #Z0622, 1in150)

5.2.4 Secondary reagent- anti-rabbit AF555 (Invitrogen, #A21428, 1in100) in anti-mouse HRP (Dako, #K4001)

5.2.5 Tertiary reagent- TSA Cy5 (PerkinElmer, #NEL745B001KT) in Source Bioscience Tris buffer (x1)(#SPGY-0108-0100) (1in100)

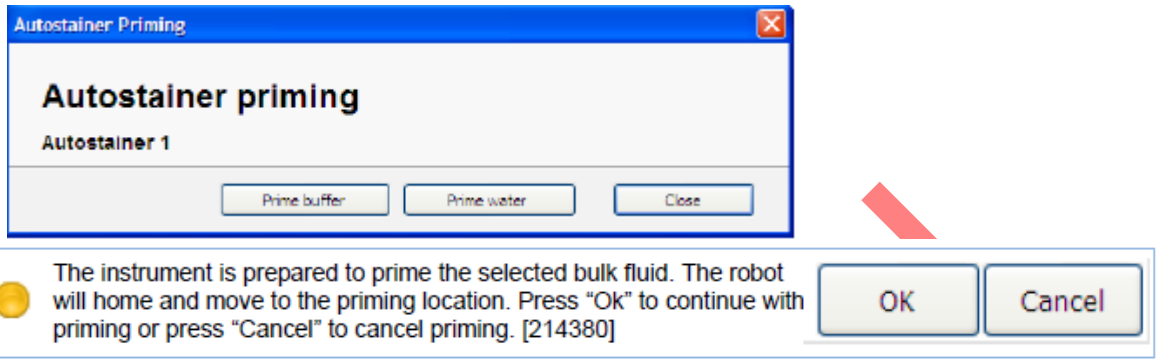
5.2.6 Counterstain-Hoechst (Invitrogen, #H3570) 1in20 in elga water

5.2.7 Prepare wash buffer (DAKO, #DM831) or equivalent wash buffer (0.1% PBST) and deionised water and make sure waste bottle is empty.

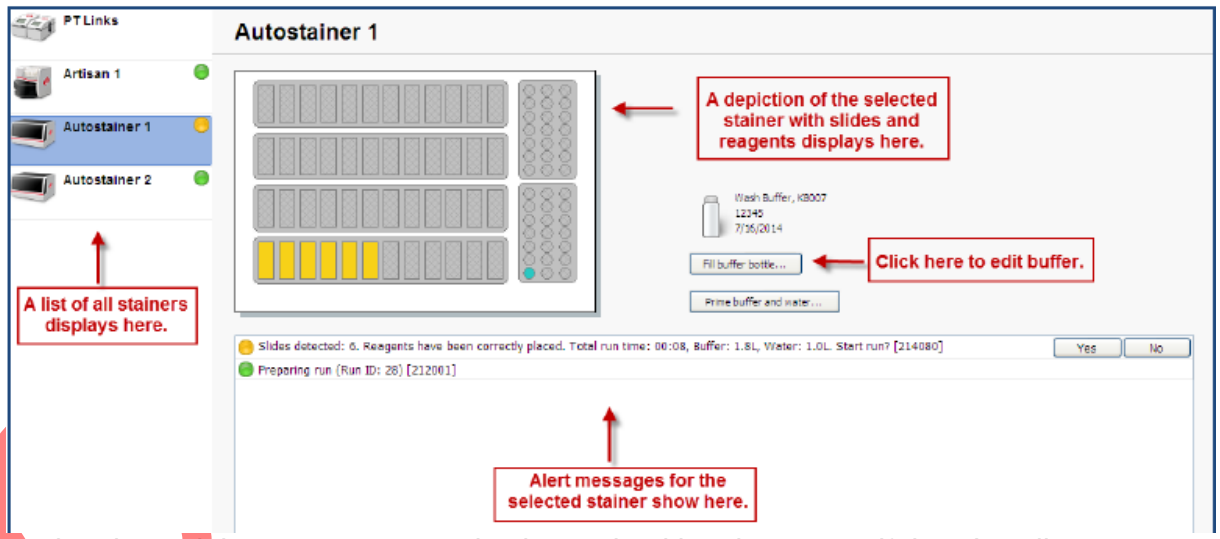
5.3 Print and apply labels for each reagent's bottle and load it to the reagent rack and the rack to the machine.

6. Wash slides in wash buffer for 5min
7. Load slides into the black slide rack on the machine and moisten slides with wash buffer. (*Note:Make sure the slide rack is straight and even.)

8. Click the **'Instrument'** tab and choose 'AS1173D0903'
   Prime the buffer and water. When the following message displays, click OK. In this step, please make sure in the buffer and water nozzles are not having any air bubbles. If there's then repeat priming the buffer and water.



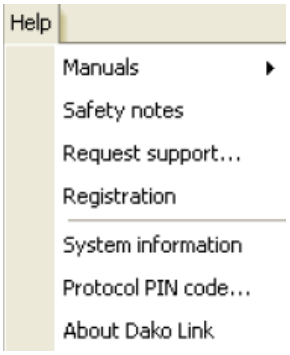
9. Starting the run in the instruments tab
   (Note: Green colour indicates normal operation message.
   Yellow indicates instrument needs attention but will not spoil slides.
   Conditions that cause yellow alerts include indications that reagents are required before the run can be processed.
   Red indicates that you need to address the problem immediately.
   Conditions that cause red alerts include system error messages.)



10. During the staining run, every 30mins keep checking the screen if there's yellow or red warnings. If so, fix the problems. Eg) Lack of reagents (yellow warning) – add more reagents)
11. End of the run, click the DONE button then the machine will purge the waste liquid.
12. Open the autostainer lid and remove completed slide racks and transfer each slide into designated wash buffer jar.
13. Dehydrate the slides in 80% alcohol for 1 min and air dry in the dark.
14. Mount slides with prolong gold anti-fade medium.
15. Scan slides in a designated scanner.
16. After scanning store slides in 2-8°C fridge.

5.0 Troubleshooting

PDF manual is available in Help



6.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

7.0 Spillages -

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

8.0 Training -

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

9.0 Related documents –

9.1 Risk assessments –COSHH RA 08
General RA 06

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

9.0 Approval and sign off –

Author:

Name: In Hwa Um

Position: PhD Student

Signature: Date:

Management Approval:

Name: Peter Mullen

Position: Research Fellow

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