

Document N	lumber: 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.



DakoLink

3. Insert slide information in New slides tab

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System Adn	ninistration Help			v						
	New slides		Workflow	-	Instruments		Completed			
New	slides									
Patient o										
Case number 4567	r:		Accession	number:	Hospital: Downtown Ho	pital 🗸				
First name:	Middle name:	Last name:	Accession	date:	Pathologist:					
John	L.	Doe	7/10/2013	2	V Dr. A	~				
Slides										
Protoco	l: lanosome HM845	Block:	Type:		reparation: D	rop zones:	Control si de	Silde notes:	Quantity:	Add slide
3.1	Add Pa		ase inf				Counterstain			
3.2				-	antibody	<b>'MC</b>		. whicł	n includes	<b>USTAN</b>
			•		' visualiz			, -		
3.3	Block-s									
3.4	Type-sl	kip								
3.5	Prepara	ation-cl	hoose	eithe	r FFPE (	or Cyt	ology			
3.6	Drop zo	ones-cl	noose	the d	rop zone	appr	opriately		Drop z	ones:
3.7	Slide n	otes-if	neces	ssary						
3.8	Quanti	ty								
3.9	Print ar	nd appl	y the s	lide l	abels					
3.10	Click 'C									
3.11	Stick th	e label	l <mark>on</mark> the	e slide	es					
. Put th	e slide(s	s) in no	on-met	tallic	rack. <u>(N</u>	o dew	vaxing a	<u>nd rehy</u>	/dration st	<u>teps</u>
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u <mark>al antig</mark>	gen re <mark>tı</mark>	ieval								
4.1 Ma	a <mark>ke u</mark> p E	DTA-T	ris 1 L	solut	tion:					
					is Base :		'g			
					TA = 0.3	37g				
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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

👌 DakoLink						
System Administration Help		_				
New slides	Workflow	Instruments	Complete	d		
Workflow	Ny Slides H&E I	HC Special Stains Re	ady for pretreatment	Pretreating	Ready for staining	Staining
To group by a column, drag its has	ader here				View: "THC default	
Slide number   Case number   Targ	et retrieval Protocol	Primary antibody Visual	lization system Zones	Instrument	Workflow	state

- 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.

A	utostainer Priming		
	Autostainer priming		
	Prime buffer Prime water Close		
0	The instrument is prepared to prime the selected bulk fluid. The robot will home and move to the priming location. Press "Ok" to continue with priming or press "Cancel" to cancel priming. [214380]	ОК	Cancel

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.)

PTLinks	Autostainer 1
Artisan 1 Autostainer 1 Autostainer 2 Autostainer 2 Alist of all stainers displays here.	A depiction of the selected stainer with slides and reagents displays here. Weth Buffer, K8007 123-6 7/5/014 Fillbuffer botte ← Click here to edit buffer. Prme buffer and mater Slides detected: 6. Reegents have been correctly placed. Total run time: 00:08, Buffer: 1.8L, Water: 1.0L. Start run? [214080] Yes No
	Alert messages for the selected stainer show here.

- 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

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	Manuals	Þ
	Safety notes	
	Request support	
	Registration	
	System information	
	Protocol PIN code	
	About Dako Link	

### 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:
Managemer	t 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