

## InsituPro VS

### Template protocol ICC 1

Date: May 2004

Configuration: 96 medium sized baskets

#### Description:

This protocol can be used as a template for seamless adaptation of short manual *in situ* immunostains. The template includes incubation with primary antibody and secondary antibody as well as all washing steps between and after these two incubations. Most steps are performed below ambient temperature. Please keep in mind to set temperature 1 on the thermocontroller to the temperature you like to reach.

All fine tuning parameters like pipetting speed etc. are set to their optimum. Therefore you can adapt your manual method with just a few clicks.

You can not reach incubation times below 40 minutes when working on the complete specimen tray filled with 96 baskets. For steps that need a shorter incubation time like proteinase K, please use the protocol ICC 2 as a template.

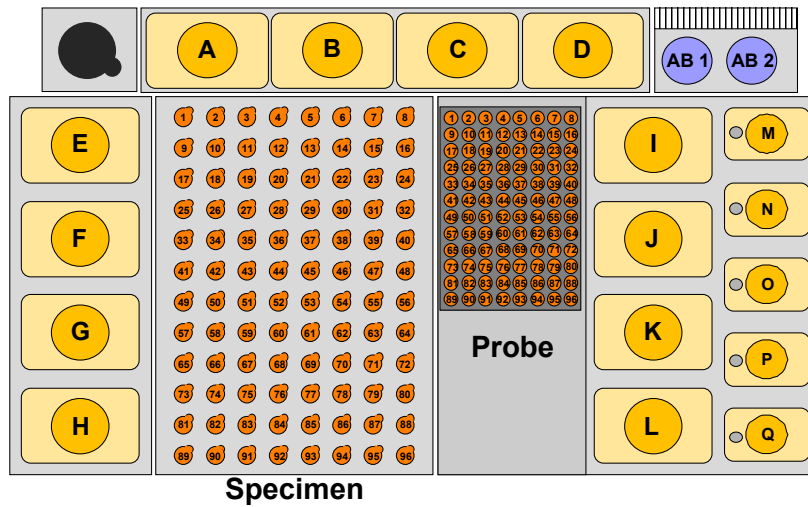
Step No.	Task	Time	Action	Proceeding
1	SetTempReg		T0 (OFF)	
2	PrimeNeedle		12000 / 12000 $\mu$ l	
3	IncubateVT	40 min	700 $\mu$ l PBST->Specimen <b>2x</b>	Wash with PBST
4	SetTempReg		TC (COOL)	Cool specimen rack for AB steps
5	IncubateVT	40 min	700 $\mu$ l TBST->Specimen	TBST Wash
6	IncubateVT	2 h	700 $\mu$ l Blocking->Specimen	Blocking
7	IncubateVT	6 h	700 $\mu$ l Probe->Specimen	Add primary antibody
8	IncubateVT	40 min	700 $\mu$ l TBST->Specimen <b>2x</b>	TBST Wash
9	IncubateVT	1 h	700 $\mu$ l TBST->Specimen <b>2x</b>	TBST Wash
10	IncubateVT	1.5 h	700 $\mu$ l TBST->Specimen	TBST Wash
11	IncubateVT	6 h	700 $\mu$ l sec. antibody->Specimen	Add secondary antibody
12	IncubateVT	1 h	700 $\mu$ l TBST->Specimen <b>2x</b>	TBST Wash
13	IncubateVT	2 h	700 $\mu$ l TBST->Specimen <b>3x</b>	TBST Wash
14	IncubateVT	2 h	700 $\mu$ l TBST->Specimen <b>3x</b>	TBST Wash
15	Pause			Wait for you
16	IncubateVT	40 min	700 $\mu$ l NTMT->Specimen <b>2x</b>	NTMT wash
17	SetTempReg		T0 (OFF)	
18	PrimeNeedle		12000 / 12000 $\mu$ l	

# Specimen and Buffer loading Form

Method: ICC 1

User: \_\_\_\_\_

Date: \_\_\_\_\_



## Buffer Loading:

Vial	Buffer	Volume	Vial	Buffer	Volume
A		0 ml	<b>J*</b>	TBST	250 ml
B		0 ml	<b>K*</b>	TBST	250 ml
C		0 ml	<b>L*</b>	TBST	250 ml
D		0 ml	M		0 ml
<b>E*</b>		250 ml	N		125 ml
<b>F*</b>		250 ml	O		125 ml
<b>G*</b>		250 ml	P		125 ml
<b>H*</b>	PBST	250 ml	<b>Q / Q2</b>	Blocking solution / NTMT	125 ml
<b>I*</b>	TBST	250 ml	<b>AB</b>	Secondary antibody	2x50 ml

Buffer printed in bold letters have to been put in during the Pause task !

Both vials on position AB (cooled rack) should be filled with antibody. The instrument needs up 75 ml antibody for the complete specimen rack!

Buffer amount can be reduced to 50 ml for positions E-H and I-L (labelled with **\***) by using the falcon adapters.