

InsituPro VS

Template protocol ICC 1, slide

Date: March 2005

Configuration: 60 slides

Description:

This protocol can be used as a template for seamless adaptation of manual *in situ* immunostains on slides. The template includes incubation with primary antibody and secondary antibody as well as all washing steps between and after these two incubations. All steps are performed at ambient temperature.

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 10 minutes when working on the complete slide tub filled with 60 slides. For steps that need a shorter incubation time like proteinase K, please use method ICC 2 as a template.

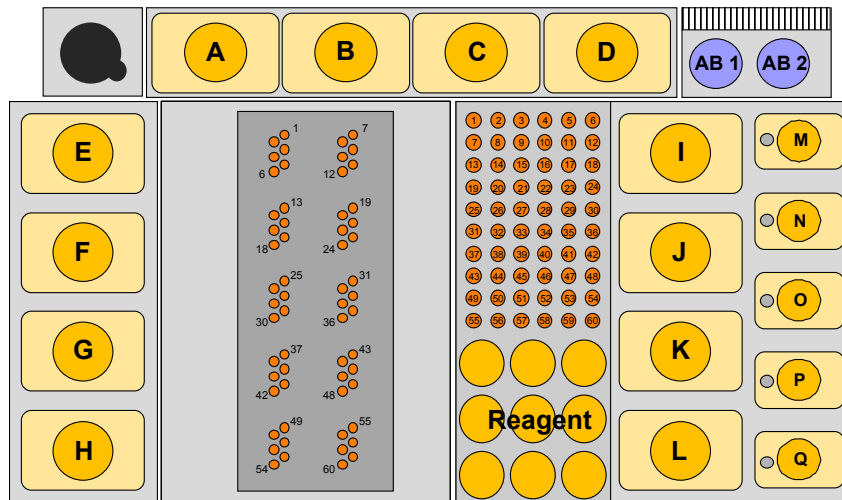
Step No.	Task	Time	Action	Proceeding
1	SetTempReg		T0 (OFF)	
2	PrimeNeedle		12000 µl	
3	PrimeTub		60000 µl	
4	IncubateTS	10 min	250 µl PBST->Slides 6x	PBST wash
5	PrimeTub		60000 µl	
6	IncubateTS	30 min	250 µl Blocking->Slides 2x	Blocking
7	PrimeTub		30000 µl	
8	IncubateTS	6 h	250 µl Probe->Slides	Primary antibody
9	PrimeTub		30000 µl	
10	IncubateTS	10 min	250 µl PBST->Slides 6x	PBST wash
11	IncubateTS	10 min	250 µl PBST->Slides 6x	PBST wash
12	PrimeTub		30000 µl	
13	IncubateTS	4 h	250µl secondary-antibody->Slides	Secondary antibody
14	PrimeTub		30000 ml	
15	IncubateTS	10 min	250 µl TBST->Slides 10x	TBST wash
16	IncubateTS	10 min	250 µl TBST->Slides 10x	TBST wash
17	IncubateTS	10 min	250 µl AP-buffer->Slides 4x	AP-buffer
18	PrimeTub		60000 µl	
19	PrimeNeedle		12000 µl	
20	SetTempReg		T0 (OFF)	

Specimen and Buffer loading Form

Method: ISH 1

User: _____

Date: _____



Buffer Loading:

Vial	Buffer	Volume
A*		
B*		
C*		
D*		
E*	PBST	
F*	PBST	
G*		
H*		
I*	TBST	
J*	TBST	

Vial	Buffer	Volume
K*		
L*		
M		
N		
O		
P	AP-buffer	
Q / Q2		
AB		
AB 1	Blocking	
AB 2	Dig-antibody	

Reagent

1		2		3	
4		5		6	
7		8		9	

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

Buffer amount can be reduced to 50 ml or 12 ml for positions A-L (labelled with *****) by using the appropriate vial adapters.