InsituPro VS

Template protocol ICC 2, slide
Date: **May 2004**
Configuration: **60 slides**
Description:

This protocol can be used as a template for seamless adaptation of manual *in situ* immunostains. The template already includes most steps of a typical immunocytochemical method like rehydration of the slides, permeation using proteinase K and postfixation. The incubation with primary antibody and secondary antibody as well as all washing steps between these two incubations, 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.

The PK treatment is pre-programmed to allow an optimization of the incubation time with just a single run. The incubation times predefined are ranging from 2 x 2 minutes to 2 x 6 minutes. 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 the subzones (Slide 1-12 e.g.) predefined.

Step No.	Task	Time	Action		Proceeding
1	SetTempReg		T0 (OFF)		
2	PrimeNeedle		12000 µl		
3	PrimeTub		60000 µl		
4	IncubateTS	10 min	250 µl 100% EtOH->Slides	**2x**	100% EtOH wash
5	IncubateTS	10 min	200 µl 95% EtOH->Slides		95% EtOH wash
6	IncubateTS	10 min	200 µl 90% EtOH->Slides		90% EtOH wash
7	IncubateTS	10 min	200 µl 80% EtOH->Slides		80% EtOH wash
8	IncubateTS	10 min	200 µl 60% EtOH->Slides		60% EtOH wash
9	IncubateTS	10 min	200 µl 50% EtOH->Slides		50% EtOH wash
10	IncubateTS	10 min	200 µl 30% EtOH->Slides		30% EtOH wash
11	IncubateTS	10 min	200 µl 30% EtOH / NaCl->Slides		30% EtOH / NaCl wash
12	IncubateTS	10 min	200 µl NaCl->Slides		NaCl wash
13	PrimeTub		60000 µl		
14	IncubateTS	10 min	250 µl PBST->Slides	**6x**	PBST wash
15	IncubateTS	2 min	250 µl Prot.K->Slide 1-12	**2x**	PK treatment first group
16	IncubateTS	2 min	250 µl Glycine->Slide 1-12	**2x**	Stop PK
17	IncubateTS	2 min	250 µl PBST->Slide 1-12	**4x**	PBST wash
18	IncubateTS	3 min	250 µl Prot.K->Slide 13-24	**2x**	PK treatment second group
19	IncubateTS	2 min	250 µl Glycine->Slide 13-24	**2x**	Stop PK
20	IncubateTS	2 min	250 µl PBST->Slide 13-24	**4x**	PBST wash
21	IncubateTS	4 min	250 µl Prot.K->Slide 25-36	**2x**	PK treatment third group
22	IncubateTS	2 min	250 µl Glycine->Slide 25-36	**2x**	Stop PK
23	IncubateTS	2 min	250 µl PBST->Slide 25-36	**4x**	PBST wash
24	IncubateTS	5 min	250 µl Prot.K->Slide 49-60	**2x**	PK treatment fourth group
25	IncubateTS	2 min	250 µl Glycine->Slide 37-48	**2x**	Stop PK
26	IncubateTS	2 min	250 µl PBST->Slide 37-48	**4x**	PBST wash
27	IncubateTS	6 min	250 µl Prot.K->Slide 49-60	**2x**	PK treatment fifth group
28	IncubateTS	2 min	250 µl Glycine->Slide 49-60	**2x**	Stop PK
29	IncubateTS	10 min	250 µl PBST->Slide 49-60	**4x**	PBST wash
30	IncubateTS	10 min	250 µl PBST->Slides	**2x**	PBST wash
31	IncubateTS	10 min	250 µl Postfix->Slides	**2x**	Postfixation
32	IncubateTS	10 min	250 µl PBST->Slides	**6x**	PBST wash
33	PrimeTub		60000 µl		
34	IncubateTS	10 min	250 µl Blocking->Slides	**2x**	Blocking

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Step No.	Task	Time	Action		Proceeding
35	PrimeTub		30000 µl		
36	IncubateTS	6 h	250 µl Probe->Slides		Primary antibody
37	PrimeTub		30000 µl		
38	IncubateTS	10 min	250 µl PBST->Slides	**4x**	PBST wash
39	IncubateTS	10 min	250 µl PBST->Slides	**4x**	PBST wash
40	PrimeTub		30000 µl		
41	IncubateTS	4 h	250µl secondary-antibody->Slides		Secondary antibody
42	PrimeTub		30000 ml		
43	IncubateTS	10 min	250 µl TBST->Slides	**10x**	TBST wash
44	IncubateTS	10 min	250 µl TBST->Slides	**10x**	TBST wash
45	IncubateTS	10 min	250 µl AP-buffer->Slides	**4x**	AP-buffer
46	PrimeTub		60000 µl		
47	PrimeNeedle		12000 µl		
48	SetTempReg		T0 (OFF)		

Specimen and Buffer loading Form

Method: ISH 2   **User:** _____   **Date:** _____
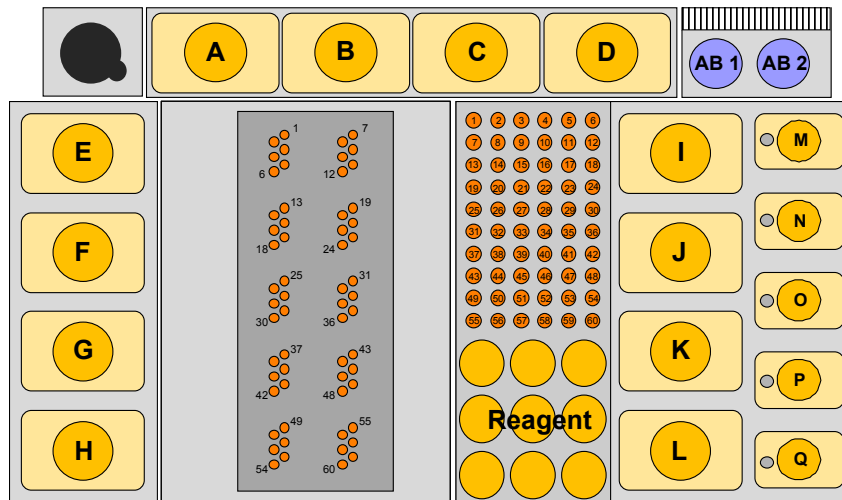
Buffer Loading:

Vial	Buffer	Volume		Vial	Buffer	Volume
A*				K*		
B*				L*		
C*				M	Proteinase K	
D*				N	Glycine	
E*	PBST			O	Postfix	
F*	PBST			P	AP-buffer	
G*				Q / Q2	100% EtOH	
H*				AB		
I*	TBST			AB 1	Blocking	
J*	TBST			AB 2	Dig-antibody	

Reagent

1	95% EtOH	**2**	90% EtOH	**3**	80% EtOH
4	60% EtOH	**5**	50% EtOH	**6**	30% EtOH
7	30% EtOH / NaCl	**8**	NaCl	**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.

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