

## InsituPro VS

### Template protocol ICC 2

Date: May 2004

Configuration: 96 small sized baskets

#### 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 specimen, 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 a temperature below ambient.

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 4 minutes to 26 minutes. 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 subzones (Basket 1-8 e.g.) predefined.

Step No.	Task	Time	Action	Proceeding
1	SetTempReg		T0 (OFF)	
2	PrimeNeedle		12000 / 12000µl	
3	IncubateVT	40 min	125 µl 50% MeOH->Specimen	First rehydration (75%)
4	IncubateVT	40 min	250 µl 50% MeOH->Specimen	Second rehydration (50%)
5	IncubateVT	40 min	125 µl PBST->Specimen	Third rehydration (25%)
6	IncubateVT	40 min	250 µl PBST->Specimen 2x	Wash with PBT
7	IncubateVT	4 min	250 µl PK solution->Basket 1-8	PK treatment first group
8	IncubateVT	5 min	250 µl Glycine->Basket	Stop PK first group
9	IncubateVT	5 min	250 µl PBST->Basket 1-8	Wash first group
10	IncubateVT	6 min	250 µl PK solution->Basket 9-16	PK treatment second group
11	IncubateVT	5 min	250 µl Glycine->Basket 9-16	Stop PK second group
12	IncubateVT	5 min	250 µl PBST->Basket 9-16	Wash second group
13	IncubateVT	8 min	250 µl PK solution->Basket 17-24	PK treatment third group
14	IncubateVT	5 min	250 µl Glycine->Basket 17-24	Stop PK third group
15	IncubateVT	5 min	250 µl PBST->Basket 17-24	Wash third group
16	IncubateVT	10 min	250 µl PK solution->Basket 25-32	PK treatment fourth group
17	IncubateVT	5 min	250 µl Glycine->Basket 25-32	Stop PK fourth group
18	IncubateVT	5 min	250 µl PBST->Basket 25-32	Wash fourth group
19	IncubateVT	12 min	250 µl PK solution->Basket 33-40	PK treatment fifth group
20	IncubateVT	5 min	250 µl Glycine->Basket 33-40	Stop PK fifth group
21	IncubateVT	5 min	250 µl PBST->Basket 33-40	Wash fifth group
22	IncubateVT	14 min	250 µl PK solution->Basket 41-48	PK treatment sixth group
23	IncubateVT	5 min	250 µl Glycine->Basket 41-48	Stop PK sixth group
24	IncubateVT	5 min	250 µl PBST->Basket 41-48	Wash sixth group
25	IncubateVT	16 min	250 µl PK solution->Basket 49-56	PK treatment seventh group
26	IncubateVT	5 min	250 µl Glycine->Basket 49-56	Stop PK seventh group
27	IncubateVT	5 min	250 µl PBST->Basket 49-56	Wash seventh group
28	IncubateVT	18 min	250 µl PK solution->Basket 57-64	PK treatment eighth group
29	IncubateVT	5 min	250 µl Glycine->Basket 57-64	Stop PK eighth group
30	IncubateVT	5 min	250 µl PBST->Basket 57-64	Wash eighth group
31	IncubateVT	20 min	250 µl PK solution->Basket 65-72	PK treatment ninth group
32	IncubateVT	5 min	250 µl Glycine->Basket 65-72	Stop PK ninth group
33	IncubateVT	5 min	250 µl PBST->Basket 65-72	Wash ninth group
34	IncubateVT	22 min	250 µl PK solution->Basket 73-80	PK treatment tenth group

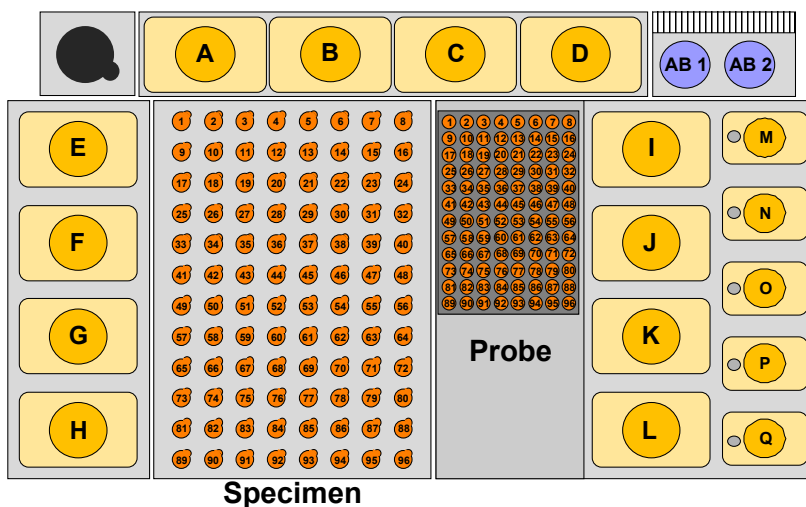
Step No.	Task	Time	Action	Proceeding
35	IncubateVT	5 min	250 µl Glycine->Basket 73-80	Stop PK tenth group
36	IncubateVT	5 min	250 µl PBST->Basket 73-80	Wash tenth group
37	IncubateVT	24 min	250 µl PK solution->Basket 81-88	PK treatment eleventh group
38	IncubateVT	5 min	250 µl Glycine->Basket 81-88	Stop PK eleventh group
39	IncubateVT	5 min	250 µl PBST->Basket 81-88	Wash eleventh group
40	IncubateVT	26 min	250 µl PK solution->Basket 89-96	PK treatment twelfth group
41	IncubateVT	5 min	250 µl Glycine->Basket 89-96	Stop PK twelfth group
42	IncubateVT	5 min	250 µl PBST->Basket 89-96	Wash twelfth group
43	IncubateVT	40 min	250 µl PBST->Specimen	Wash with PBST
44	IncubateVT	40 min	250 µl Postfix->Specimen	Postfixation
45	IncubateVT	40 min	250 µl PBST->Specimen	Wash with PBST
46	SetTempReg		TC (COOL)	Cool specimen rack for AB steps
47	IncubateVT	40 min	250 µl TBST->Specimen	TBST Wash
48	IncubateVT	2 h	250 µl Blocking->Specimen	Blocking
49	IncubateVT	6 h	250 µl Probe->Specimen	Add primary antibody
50	IncubateVT	40 min	250 µl TBST->Specimen	TBST Wash
51	IncubateVT	1 h	250 µl TBST->Specimen	TBST Wash
52	IncubateVT	1.5 h	250 µl TBST->Specimen	TBST Wash
53	IncubateVT	6 h	250 µl sec. antibody->Specimen	Add secondary antibody
54	IncubateVT	1 h	250 µl TBST->Specimen	TBST Wash
55	IncubateVT	2 h	250 µl TBST->Specimen	TBST Wash
56	IncubateVT	2 h	250 µl TBST->Specimen	TBST Wash
57	Pause			Wait for you
58	IncubateVT	40 min	250 µl NTMT->Specimen	NTMT wash
59	SetTempReg		T0 (OFF)	
60	PrimeNeedle		12000 / 12000 µl	

# Specimen and Buffer loading Form

Method: ICC 2

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

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



## Buffer Loading:

Vial	Buffer	Volume
<b>A</b>		<b>0 ml</b>
<b>B</b>		<b>0 ml</b>
<b>C</b>		<b>0 ml</b>
<b>D</b>		<b>0 ml</b>
<b>E*</b>	50% MeOH	<b>250 ml</b>
<b>F*</b>	PBST	<b>250 ml</b>
<b>G*</b>	PBST	<b>250 ml</b>
<b>H*</b>	PBST	<b>250 ml</b>
<b>I*</b>	TBST	<b>250 ml</b>

Vial	Buffer	Volume
<b>J*</b>	TBST	<b>250 ml</b>
<b>K*</b>	TBST	<b>250 ml</b>
<b>L*</b>	TBST	<b>250 ml</b>
<b>M</b>		<b>0 ml</b>
<b>N</b>	Proteinase K solution	<b>125 ml</b>
<b>O</b>	Glycine buffer	<b>125 ml</b>
<b>P</b>	Postfix solution	<b>125 ml</b>
<b>Q / Q2</b>	Blocking solution / NTMT	<b>125 ml</b>
<b>AB</b>	Secondary antibody	<b>2x50 ml</b>

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

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