

## InsituPro VS

### Template protocol ISH 2, slide

Date: May 2004

Configuration: 60 slides

#### Description:

This protocol can be used as a template for seamless adaptation of manual *in situ* hybridization protocols. It already includes most steps of a typical *in situ* hybridization method like rehydration of the slides, permeation using proteinase K and postfixation. The prehybridization and hybridization steps as well as the post hybridization washes are performed at elevated 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 <b>2x</b>	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->Slides6x	PBST wash
15	IncubateTS	2 min	250 µl Prot.K->Slide 1-12 <b>2x</b>	PK treatment first group
16	IncubateTS	2 min	250 µl Glycine->Slide 1-12 <b>2x</b>	Stop PK
17	IncubateTS	2 min	250 µl PBST->Slide 1-12 <b>4x</b>	PBST wash
18	IncubateTS	3 min	250 µl Prot.K->Slide 13-24 <b>2x</b>	PK treatment second group
19	IncubateTS	2 min	250 µl Glycine->Slide 13-24 <b>2x</b>	Stop PK
20	IncubateTS	2 min	250 µl PBST->Slide 13-24 <b>4x</b>	PBST wash
21	IncubateTS	4 min	250 µl Prot.K->Slide 25-36 <b>2x</b>	PK treatment third group
22	IncubateTS	2 min	250 µl Glycine->Slide 25-36 <b>2x</b>	Stop PK
23	IncubateTS	2 min	250 µl PBST->Slide 25-36 <b>4x</b>	PBST wash
24	IncubateTS	5 min	250 µl Prot.K->Slide 49-60 <b>2x</b>	PK treatment fourth group
25	IncubateTS	2 min	250 µl Glycine->Slide 37-48 <b>2x</b>	Stop PK
26	IncubateTS	2 min	250 µl PBST->Slide 37-48 <b>4x</b>	PBST wash
27	IncubateTS	6 min	250 µl Prot.K->Slide 49-60 <b>2x</b>	PK treatment fifth group
28	IncubateTS	2 min	250 µl Glycine->Slide 49-60 <b>2x</b>	Stop PK
29	IncubateTS	10 min	250 µl PBST->Slide 49-60 <b>4x</b>	PBST wash
30	IncubateTS	10 min	250 µl PBST->Slides <b>2x</b>	PBST wash
31	IncubateTS	10 min	250 µl Postfix->Slides <b>2x</b>	Postfixation
32	IncubateTS	10 min	250 µl PBST->Slides <b>6x</b>	PBST wash
33	PrimeTub		60000 µl	
34	SetTempReg		T2 (HIGH)	

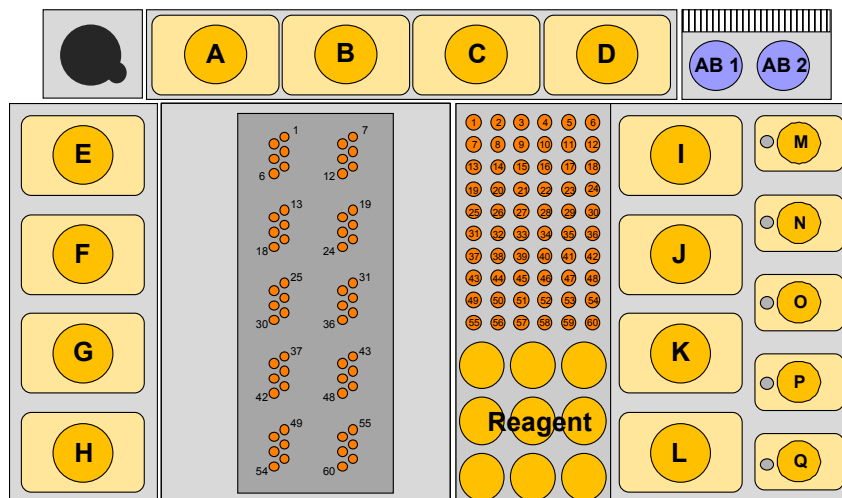
Step No.	Task	Time	Action	Proceeding
35	IncubateTS	10 min	250 µl PBST->Slides	Wash with PBST
36	IncubateTS	10 min	200µl Hyb.-Mix / PBST->Slides	Wash with Hyb.-Mix / PBST
37	IncubateTS	10 min	250 µl Hyb.-Mix->Slides <b>2x</b>	Wash with Hyb.-Mix
38	IncubateTS	1.5 h	250 µl Hyb.-Mix->Slides <b>2x</b>	Prehybridization
39	PrimeTub		30000 µl	
40	IncubateTS	12 h	250 µl Probe->Slides	Hybridization
41	PrimeTub		30000 µl	
42	IncubateTS	10 min	250 µl Wash 1->Slides <b>6x</b>	Wash 1
43	IncubateTS	10 min	250 µl Wash 2->Slides <b>6x</b>	Wash 2
44	IncubateTS	10 min	250 µl Wash 3->Slides <b>6x</b>	Wash 3
45	SetTempReg		T0 (OFF)	
46	PrimeTub		30000 µl	
47	IncubateTS	30 min	250 µl TBST->Slides <b>2x</b>	TBST wash
48	IncubateTS	30 min	250 µl Blocking->Slides <b>2x</b>	Blocking
49	PrimeTub		30000 ml	
50	IncubateTS	2 h	250µl Dig-antibody->Slides	Antibody
51	PrimeTub		30000 ml	
52	IncubateTS	10 min	250 µl TBST->Slides <b>10x</b>	TBST wash
53	IncubateTS	10 min	250 µl TBST->Slides <b>10x</b>	TBST wash
54	IncubateTS	10 min	250 µl AP-buffer->Slides <b>4x</b>	AP-buffer
55	PrimeTub		60000 µl	
56	PrimeNeedle		12000 µl	
57	SetTempReg		T0 (OFF)	

# Specimen and Buffer loading Form

Method: ISH 2

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

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



## Buffer Loading:

Vial	Buffer	Volume
<b>A*</b>	Hyb.-Mix	
<b>B*</b>	Wash 1	
<b>C*</b>	Wash 2	
<b>D*</b>	Wash 3	
<b>E*</b>		
<b>F*</b>		
<b>G*</b>		
<b>H*</b>		
<b>I*</b>	PBST	
<b>J*</b>	PBST	

Vial	Buffer	Volume
<b>K*</b>	TBST	
<b>L*</b>	TBST	
<b>M</b>	Proteinase K	
<b>N</b>	Glicine	
<b>O</b>	Postfix	
<b>P</b>	AP-buffer	
<b>Q / Q2</b>	100% EtOH	
<b>AB</b>		
<b>AB 1</b>	Blocking	
<b>AB 2</b>	Dig-antibody	

## Reagent

<b>1</b>	95% EtOH	<b>2</b>	90% EtOH	<b>3</b>	80% EtOH
<b>4</b>	60% EtOH	<b>5</b>	50% EtOH	<b>6</b>	30% EtOH
<b>7</b>	30% EtOH / NaCl	<b>8</b>	NaCl	<b>9</b>	Hyb-Mix / PBST

Buffer printed in bold letters have to be 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.