

## InsituPro VS

### Template protocol ISH 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* hybridization protocols. The template includes prehybridization and hybridization steps as well as the post hybridization washes, all performed at elevated temperature. The incubation with DIG antibody and intensive washing steps are included as well.

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 ISH 2 as a template.

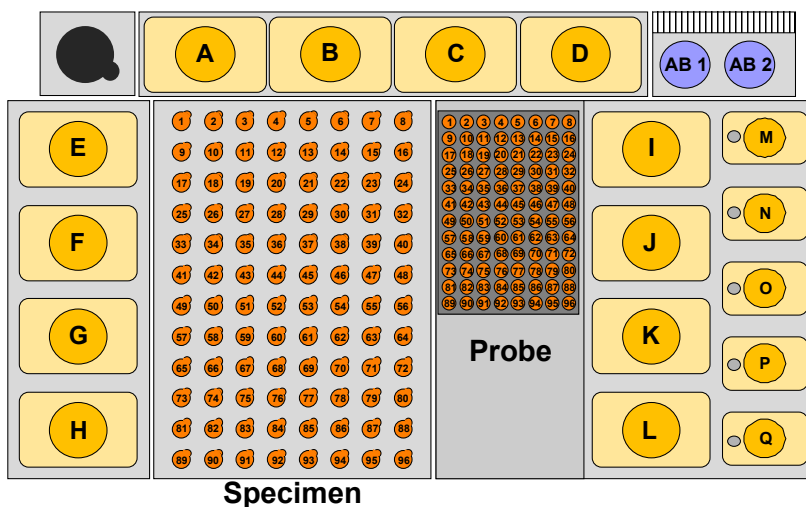
Step No.	Task	Time	Action	Proceeding
1	SetTempReg		T0 (OFF)	
2	PrimeNeedle		12000 / 12000 µl	
3	IncubateVT	40 min	700 µl PBST->Specimen <b>2x</b>	Wash with PBST
4	IncubateVT	40 min	350 µl Hyb.-Mix->Specimen	Wash with PBST/Hyb.-Mix 1:1
5	SetTempReg		T2 (HIGH)	
6	IncubateVT	40 min	700 µl Hyb.-Mix->Specimen	Wash with Hyb.Mix
7	IncubateVT	2 h	700 µl Hyb.-Mix->Specimen	Prehybridization
8	IncubateVT	12 h	700 µl Probe->Specimen	Hybridization
9	IncubateVT	40 min	700 µl Wash 1->Specimen	Posthybwash 1
10	IncubateVT	1 h	700 µl Wash 1->Specimen <b>2x</b>	Posthybwash 2
11	IncubateVT	40 min	700 µl Wash 2->Specimen	Posthybwash 2
12	IncubateVT	1 h	700 µl Wash 2->Specimen <b>2x</b>	Posthybwash 2
13	IncubateVT	40 min	700 µl Wash 3->Specimen	Posthybwash 3
14	IncubateVT	1 h	700 µl Wash 3->Specimen <b>2x</b>	Posthybwash 3
15	SetTempReg		T0 (OFF)	
16	Wait	20 min		
17	SetTempReg		TC (COOL)	
18	IncubateVT	40 min	700 µl TBST->Specimen <b>2x</b>	TBST Wash
19	IncubateVT	2 h	700 µl Blocking->Specimen	Blocking
20	IncubateVT	6 h	700 µl DIG antibody->Specimen	DIG antibody
21	SetTempReg		T0 (OFF)	
22	IncubateVT	40 min	700 µl TBST->Specimen	TBST Wash
23	IncubateVT	1 h	700 µl TBST->Specimen <b>3x</b>	TBST Wash
24	IncubateVT	1.5 h	700 µl TBST->Specimen	TBST Wash
25	IncubateVT	1.5 h	700 µl TBST->Specimen <b>2x</b>	TBST Wash
26	IncubateVT	2 h	700 µl TBST->Specimen <b>2x</b>	TBST Wash
27	Pause		Wait for you	
28	IncubateVT	40 min	600 µl NTMT->Specimen <b>2x</b>	NTMT wash
29	SetTempReg		T0 (OFF)	
30	PrimeNeedle		12000 / 12000 µl	

# Specimen and Buffer loading Form

Method: ISH 1

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

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



## Buffer Loading:

Vial	Buffer	Volume
<b>A</b>	Hyb.-Mix	<b>250 ml</b>
<b>B</b>	Wash 1	<b>250 ml</b>
<b>C</b>	Wash 2	<b>250 ml</b>
<b>D</b>	Wash 3	<b>250 ml</b>
<b>E*</b>		<b>250 ml</b>
<b>F*</b>		<b>250 ml</b>
<b>G*</b>		<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>125 ml</b>
<b>N</b>		<b>125 ml</b>
<b>O</b>		<b>125 ml</b>
<b>P</b>		<b>125 ml</b>
<b>Q</b>	Blocking solution / NTMT	<b>125 ml</b>
<b>AB</b>	DIG antibody	<b>2x50 ml</b>

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

Both vials on position AB (cooled rack) should be filled with antibody. The instrument needs up to 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.