

InsituPro VS

Template protocol ISH 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* hybridization protocols. It already includes most steps of a typical *in situ* hybridization method like rehydration of the specimen, bleaching with hydrogenperoxide, 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 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 / 12000 µl	
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 PBST
7	IncubateVT	40 min	250 µl 6% H ₂ O ₂ ->Specimen	Bleach with Hydrogenperoxide
8	IncubateVT	40 min	250 µl PBST->Specimen 2x	Wash with PBT
9	IncubateVT	4 min	250 µl PK solution->Basket 1-8	PK treatment first group
10	IncubateVT	5 min	250 µl Glycine->Basket 1-8	Stop PK first group
11	IncubateVT	5 min	250 µl PBST->Basket 1-8	Wash first group
12	IncubateVT	6 min	250 µl PK solution->Basket 9-16	PK treatment second group
13	IncubateVT	5 min	250 µl Glycine->Basket 9-16	Stop PK second group
14	IncubateVT	5 min	250 µl PBST->Basket 9-16	Wash second group
15	IncubateVT	8 min	250 µl PK solution->Basket 17-24	PK treatment third group
16	IncubateVT	5 min	250 µl Glycine->Basket 17-24	Stop PK third group
17	IncubateVT	5 min	250 µl PBST->Basket 17-24	Wash third group
18	IncubateVT	10 min	250 µl PK solution->Basket 25-32	PK treatment fourth group
19	IncubateVT	5 min	250 µl Glycine->Basket 25-32	Stop PK fourth group
20	IncubateVT	5 min	250 µl PBST->Basket 25-32	Wash fourth group
21	IncubateVT	12 min	250 µl PK solution->Basket 33-40	PK treatment fifth group
22	IncubateVT	5 min	250 µl Glycine->Basket 33-40	Stop PK fifth group
23	IncubateVT	5 min	250 µl PBST->Basket 33-40	Wash fifth group
24	IncubateVT	14 min	250 µl PK solution->Basket 41-48	PK treatment sixth group
25	IncubateVT	5 min	250 µl Glycine->Basket 41-48	Stop PK sixth group
26	IncubateVT	5 min	250 µl PBST->Basket 41-48	Wash sixth group
27	IncubateVT	16 min	250 µl PK solution->Basket 49-56	PK treatment seventh group
28	IncubateVT	5 min	250 µl Glycine->Basket 49-56	Stop PK seventh group
29	IncubateVT	5 min	250 µl PBST->Basket 49-56	Wash seventh group

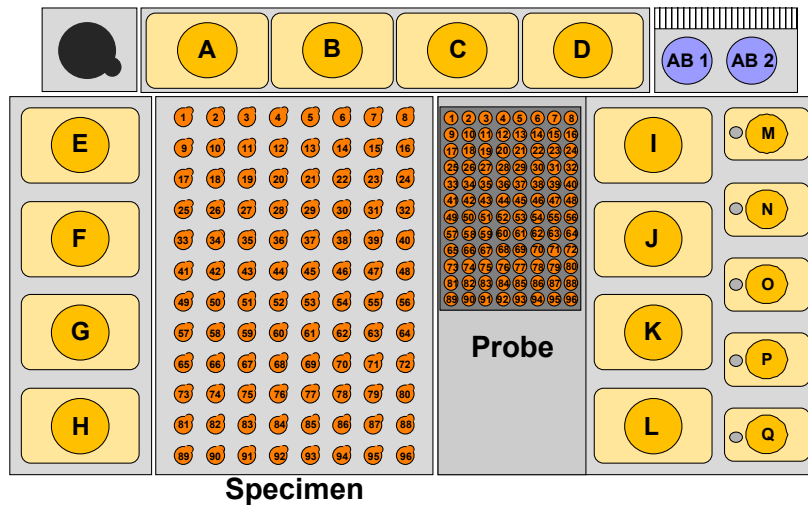
Step No.	Task	Time	Action	Proceeding
30	IncubateVT	18 min	250 µl PK solution->Basket 57-64	PK treatment eighth group
31	IncubateVT	5 min	250 µl Glycine->Basket 57-64	Stop PK eighth group
32	IncubateVT	5 min	250 µl PBST->Basket 57-64	Wash eighth group
33	IncubateVT	20 min	250 µl PK solution->Basket 65-72	PK treatment ninth group
34	IncubateVT	5 min	250 µl Glycine->Basket 65-72	Stop PK ninth group
35	IncubateVT	5 min	250 µl PBST->Basket 65-72	Wash ninth group
36	IncubateVT	22 min	250 µl PK solution->Basket 73-80	PK treatment tenth group
37	IncubateVT	5 min	250 µl Glycine->Basket 73-80	Stop PK tenth group
38	IncubateVT	5 min	250 µl PBST->Basket 73-80	Wash tenth group
39	IncubateVT	24 min	250 µl PK solution->Basket 81-88	PK treatment eleventh group
40	IncubateVT	5 min	250 µl Glycine->Basket 81-88	Stop PK eleventh group
41	IncubateVT	5 min	250 µl PBST->Basket 81-88	Wash eleventh group
42	IncubateVT	26 min	250 µl PK solution->Basket 89-96	PK treatment twelfth group
43	IncubateVT	5 min	250 µl Glycine->Basket 89-96	Stop PK twelfth group
44	IncubateVT	5 min	250 µl PBST->Basket 89-96	Wash twelfth group
45	IncubateVT	40 min	250 µl PBST->Specimen	Wash with PBST
46	IncubateVT	40 min	250 µl Postfix->Specimen	Postfixation
47	IncubateVT	40 min	250 µl PBST->Specimen 2x	Wash with PBST
48	IncubateVT	40 min	125 µl Hyb.-Mix->Specimen	Wash with PBST/Hyb.-Mix 1:1
49	SetTempReg		T2 (HIGH)	
50	IncubateVT	40 min	250 µl Hyb.-Mix->Specimen	Wash with Hyb.Mix
51	IncubateVT	2 h	250 µl Hyb.-Mix->Specimen	Prehybridization
52	IncubateVT	12 h	250 µl Probe->Specimen	Hybridization
53	IncubateVT	40 min	250 µl Wash 1->Specimen	Posthybwash 1
54	IncubateVT	1 h	250 µl Wash 1->Specimen 2x	Posthybwash 2
55	IncubateVT	40 min	250 µl Wash 2->Specimen	Posthybwash 2
56	IncubateVT	1 h	250 µl Wash 2->Specimen 2x	Posthybwash 2
57	IncubateVT	40 min	250 µl Wash 3->Specimen	Posthybwash 3
58	IncubateVT	1 h	250 µl Wash 3->Specimen 2x	Posthybwash 3
59	SetTempReg		T0 (OFF)	
60	Wait	20 min		
61	SetTempReg		TC (COOL)	
62	IncubateVT	40 min	250 µl TBST->Specimen 2x	TBST Wash
63	IncubateVT	2 h	250 µl Blocking->Specimen	Blocking
64	IncubateVT	6 h	250 µl DIG antibody->Specimen	DIG antibody
65	SetTempReg		T0 (OFF)	
66	IncubateVT	40 min	250 µl TBST->Specimen	TBST Wash
67	IncubateVT	1 h	250 µl TBST->Specimen 3x	TBST Wash
68	IncubateVT	1.5 h	250 µl TBST->Specimen	TBST Wash
69	IncubateVT	1.5 h	250 µl TBST->Specimen 2x	TBST Wash
70	IncubateVT	2 h	250 µl TBST->Specimen 2x	TBST Wash
71	Pause		Wait for you	
72	IncubateVT	40 min	250 µl NTMT->Specimen 2x	NTMT wash
73	SetTempReg		T0 (OFF)	
74	PrimeNeedle		12000 / 12000 µl	

Specimen and Buffer loading Form

Method: ISH 2

User: _____

Date: _____



Buffer Loading:

Vial	Buffer	Volume
A	Hyb.-Mix	250 ml
B	Wash 1	250 ml
C	Wash 2	250 ml
D	Wash 3	250 ml
E*	50% MeOH	250 ml
F*	PBST	250 ml
G*	PBST	250 ml
H*	PBST	250 ml
I*	TBST	250 ml

Vial	Buffer	Volume
J*	TBST	250 ml
K*	TBST	250 ml
L*	TBST	250 ml
M	6% H ₂ O ₂	125 ml
N	Proteinase K solution	125 ml
O	Glycine buffer	125 ml
P	Postfix solution	125 ml
Q/Q2	Blocking solution / NTMT	125 ml
AB	DIG antibody	2x50 ml

Buffer printed in bold letters have to be 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.