

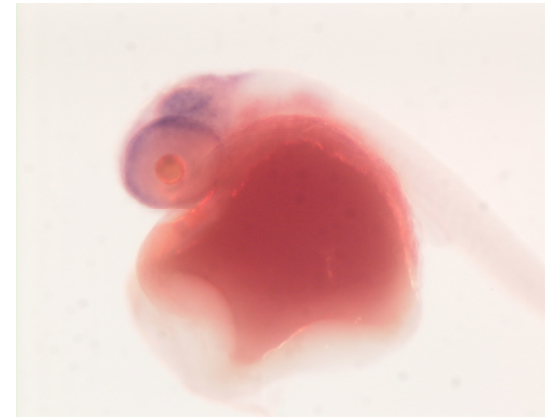
InsituPro UP Protocol: Medaka 1

Date: 01.2006

System Configuration: 30 small baskets

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Medaka,

Description

This protocol is used for Medaka embryos (stage ?), which have been rehydrated, permeated by PK and postfixed. The specimen are loaded in 200µl of PTw for each basket each. The method automates all steps from prehybridization of the embryos till the incubation in alkaline phosphatase buffer. It is optimized for the InsituPro small basket upgrade work area.

Incubation with staining solution is performed outside the instrument.

Method Listing

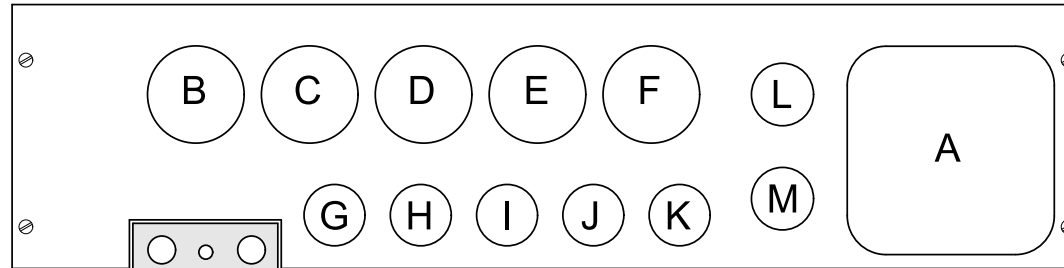
Step	Task	Time	Aliquoting	Thats what it means	Elapsed Time
1	SetTempReg		0 (OFF)		↓ 1.2 h
2	PrimeNeedle		12000		
3	IncubateVT	15 min	200 PTW->Specimen	Wash with PTw	
4	IncubateVT	15 min	100 Hyb-mix->Specimen	Wash Hyb.-Mix / PTw	
5	SetTempReg		(HIGH)		
6	IncubateVT	1 h	200 Hyb-mix->Specimen	Prehybridization	
7	IncubateVT	8 h	200 Probe->Specimen	Hybridization	
8	IncubateVT	8 min	200 2xSSC/50%FA->Specimen	5x Posthybwash 1	
9	IncubateVT	10 min	200 2xSSC/50%FA->Specimen	Posthybwash 1	
10	IncubateVT	8 min	200 2xSSC->Specimen	5x Posthybwash 2	
11	IncubateVT	10 min	200 2xSSC->Specimen	Posthybwash 2	
12	IncubateVT	8 min	200 0.2xSSC->Specimen	5x Posthybwash 3	
13	IncubateVT	10 min	200 0.2xSSC->Specimen	Posthybwash 3	
14	SetTempReg		T0 (OFF)		
15	Wait	30 min		Coll down thermorack	
16	IncubateVT	10 min	200 PTW->Specimen	2x Wash with PTw	
17	IncubateVT	1 h	200 Blocking soln->Specimen	Blocking	
18	IncubateVT	4 h	200 Dig antibody->Specimen	DIG antibody	
19	IncubateVT	8 min	200 PTW->Specimen	8x PTw	
20	IncubateVT	8 min	200 Prestaining buffer->Specimen	2x Prestain Buffer	
21	Pause		Add staining buffer to position G and press return	MANUAL STEP !	
22	IncubateVT	8 min	200 Staining buffer->Specimen	2x Staining buffer	↓ 19.4
23	PrimeNeedle		12000		
24	SetTempReg		T0 (OFF)		

Buffer Loading

Method: Medaka

User:

Date:



Position	Buffer	Amount
A / A2	PTw	
B	Hyb.-Mix	
C	2xSSC / 50% FA	
D	2xSSC	
E	0.2xSSC	
F	Prestaining buffer	
G	Staining buffer	

Position	Buffer	Amount
H	-	
I	-	
J	-	
K	-	
L	Blocking solution	
M	DIG antibody	
Probe		

Buffer printed in bold letters have to be put in during the wait for key task !

Buffers

Buffer : PTw	pH : 7.5
Substance	Concentration
Na ₂ HPO ₄ x 2H ₂ O	7 mM
KH ₂ PO ₄	1,5 mM
NaCl	137 mM
KCl	2,7 mM
Tween 20	0,1%

Buffer : Hybmix	pH : 4.5
Substance	Concentration
Formamide (Flika, ultra pure)	50%
SSC	5x
tRNA	5mg / ml
Heparine	50 µg / ml
Tween 20	0.1 %

Buffer : Posthybwash 1	pH : 7.5
Substance	Concentration
Formamide	50%
SSC	2x
Tween 20	0.1 %

Buffer : Posthybwash 2	pH : 7.5
Substance	Concentration
SSC	2x
Tween 20	0.1 %

Buffer : Posthybwash 3	pH : 7.5
Substance	Concentration
SSC	0.2x
Tween 20	0.1 %

Buffer : Blocking buffer	pH : 7.5
Substance	Concentration
Malate	200 mM
NaCl	300 mM
Tween 20	0.1 %
Blocking reagent (Roche) 10%	40 % v/v

Buffer : DIG AB	pH : 7.5
Substance	Concentration
Anti-DIG FAB	1:2000
. . .in Blocking buffer	

Buffer : Prestaining	pH : 9.5
Substance	Concentration
TrisCl	100 mM
NaCl	100 mM
Tween 20	0.1 %

Buffer : Staining	pH : 9.5
Substance	Concentration
TrisCl	100 mM
NaCl	100 mM
MgCl ₂	50 mM
Tween 20	0.1 %

Additional Tipps&Trick:

- PTw is sterile filtered through a 0.2 µm nitrocellulose filter prior to use