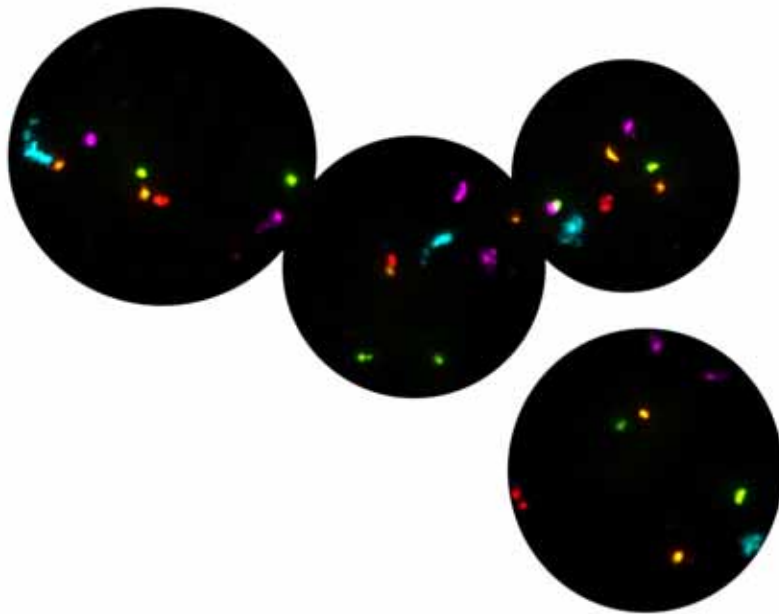


# ***One Cell Systems, Inc.***

## **Oligo-FISH probes Instruction Manual**

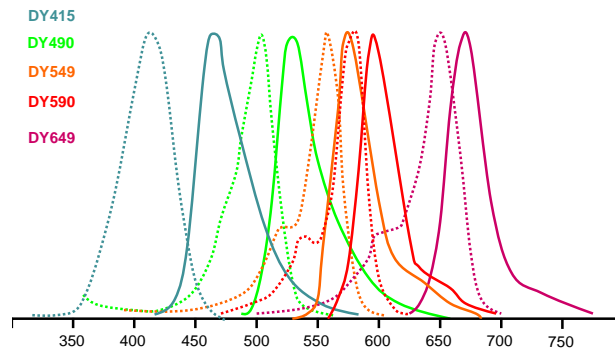




## Reagents Provided

Reagent	Volume	Composition	Storage
Probe Cocktail	50 $\mu$ L	Fluor labeled oligonucleotides in water	+4°C in the dark
Hybridization Buffer	50 $\mu$ L	Dextran sulfate, formamide and SSC	+4°C in the dark

## Fluors Used for Labeling



## Reagents Required but not Provided

Reagent	Cat. #	Supplier
20xSSC	S6639	Sigma
*Sodium Citrate	S1804	Sigma
*NaCl	S7653	Sigma
**Formamide	F7508	Sigma
SDS 10% solution	L4522	Sigma
p-phenylenediamino dihydrochloride	P1519	Sigma
DAPI	D9542	Sigma
10xPBS	14200-075	Invitrogen
NaOH	221465	Sigma
Glycerol	G5516	Sigma
Ethanol 100%		

\*If you purchase prepared 20xSSC, these items are not required.

\*\*If you prefer to use a programmable hot plate, these items are not required.

## Preparing Solutions for Performing Oligo-FISH Assay

### Aliquoting Formamide

Formamide is sensitive to light, temperature and glass containers. We strongly recommend preparing aliquots.

1. Aliquot 35mL of Formamide in 50mL Falcon tubes and keep at -20°C protected from the light.

### 20xSSC (0.3M Sodium Citrate, 3M NaCl)

If you are purchasing 20xSSC premixed, proceed to step 5.

2. To prepare 1L of 20xSSC, mix in a flask: 88.2g of Sodium Citrate, 175.3g of NaCl and 800mL of water. Agitate continuously until completely dissolved.

3. Adjust pH to 7 and add water until total volume equals 1L. Although this solution can be kept at room temperature, it should be replaced if salt crystals start forming at the top of the bottle.
4. To prepare **2xSSC**, dilute 1:10 20xSSC in water (ie: 50mL 20xSSC into 450mL of water).

#### **Denaturation Solution (70% Formamide, 2xSSC)**

If you prefer to use a programmable hot plate, proceed to step 8.

5. Unfreeze one aliquot of 35mL of Formamide.
6. Add 5mL of 20xSSC into the same tube and add water until 50mL total volume.
7. Pour the Denaturation Solution into a glass coplin jar and place it into a 72°C water bath. Allow the temperature to stabilize for at least 30 minutes before use.

#### **Washing Solution (0.2xSSC, 0.1%SDS)**

8. Into a 50mL Falcon tube, add 0.5mL of 20xSSC and 0.5mL of 10% SDS. Then add water to increase total volume to 50mL.
9. Pour the Washing Solution into a coplin jar and place it in a 50°C water bath. Allow the temperature to stabilize for at least 20 minutes before use.

#### **Alternative Washing Solution with NP-40 (0.2xSSC, 0.3%NP-40)**

8. Into a 50mL Falcon tube add 0.5mL of 20xSSC and 150µL of NP-40. Then add water to increase total volume to 50mL.
9. Pour the Washing Solution into a coplin jar and place it in a 50°C water bath. Allow the temperature to stabilize for at least 20min before use.

### 10N NaOH Solution

10. Weigh 20g of NaOH and pour it into a 50mL Falcon tube. (Handle NaOH carefully since it is highly corrosive and can burn the skin)
11. Slowly add 50mL of water. Since dilution of NaOH is exothermic, agitate slowly and cool the tube in ice until NaOH is completely dissolved. This solution is very stable and can be kept at room temperature for up to 1 year.

### Antifade Solution

12. Weigh 100mg of p-phenylenediamine dihydrochloride and pour it into a 50mL Falcon tube. Protect the tube from light with aluminum foil.
13. Add 8mL of 10XPBS into the tube and mix well.
14. Adjust the pH to 8 adding drops of 10N NaOH. (Handle NaOH carefully since it is highly corrosive and can burn the skin)
15. Adjust the volume of the solution to 10mL adding 10xPBS
16. Add this solution to 90mL of glycerol.
17. Mix well and prepare 1mL aliquots in 1.5mL micro centrifuge tubes. Keep the aliquots at -20°C protected from the light for no longer than 3 months. (If the solution turns brown, it is no longer effective and should be discarded)
18. After removing aliquots from -20°C, they should be used the same day. If DNA will be stained with DAPI, proceed to the next steps; otherwise, this Antifade Solution can be used directly on the fluorescence slides after the Oligo-FISH Assay Procedure.

### DAPI 200ng/μL Solution

19. Mix 1mg of DAPI with 5mL of water.

20. Aliquot in small amounts (~10 $\mu$ L) in 1.5mL micro centrifuge tubes and keep at -20°C protected from the light. (This solution is very stable and can be used for up to 1 year).
21. Add 1 $\mu$ L of DAPI 200ng/ $\mu$ L in 1mL antifade, mix well since the solution is very viscous. This antifade solution should be prepared daily.

## Oligo-FISH Assay Procedure

### Preparing the FISH Mix

Depending on the hybridization area, different volumes of FISH Mix should be used:

Cover slips	FISH Mix volume
Square 22x22mm	10 $\mu$ L
Square 18x18mm	7 $\mu$ L
Square 12x12mm	3 $\mu$ L
Round 22mm diameter	8 $\mu$ L
Round 18mm diameter	5 $\mu$ L
Round 12mm diameter	3 $\mu$ L
Other sizes	Volume= $\text{mm}^2 \times 0.02\mu\text{L}$

1. Mix equal volumes of Hybridization Buffer and Probe Cocktail to obtain the hybridization mix ready for use. ie: 1.5 $\mu$ L Hybridization buffer + 1.5 $\mu$ L Probe Cocktail for a working volume of 3 $\mu$ L.

### Instructions for Separate Slide Denaturation Procedure

2. Denature each slide in Denaturation Solution at 72°C for 2 minutes.
3. Dehydrate the slides in a cold ethanol series: 70%, 80%, 90% and 100%, 2 minutes each.
4. Air dry the slides.

5. Drop the FISH mix on the cells on the slide.
6. Cover with the appropriate cover slip.
7. Place at 37°C for 5 minutes (hybridization step).
8. Place slides in 2xSSC for 5 minutes and agitate to remove cover slips.
9. Place slides in Wash Solution at 50°C for 2 minutes, gently agitate initially for 30 seconds.
10. Place slides in 2xSSC at room temperature.
11. Add antifade with DAPI and cover with a 50mm x 22mm cover slip (#1 thickness).

#### **Using a Programmable Hot Plate**

2. Drop the FISH mix on the cells on the slide.
3. Cover with the appropriate cover slip.
4. Preheat the hot plate at 85°C. Place the slides on the hot plate set at 85°C for 5 minutes (denaturation step), then 37°C for 5 minutes (hybridization step).
5. Place slides in 2xSSC for 5 minutes and agitate to remove cover slips.
6. Place slides in Wash Solution (0.2xSSC, 0.1% SDS) at 50°C for 2 minutes, gently agitate initially for 30 seconds.
7. Place slides in 2xSSC at room temperature.
8. Add antifade with DAPI and cover with a 50mm x 22mm cover slip (#1 thickness).

## Optional Slide Treatments

For some tissues or cell types, treating the slides prior to hybridization may result in superior results. We recommend any of the following treatments:

## Reagents Required but not Provided

Reagent	Cat. #	Provider
RNase	R6513	Sigma
Pepsin	P7000	Sigma
HCl 37%	320331	Sigma
MgCl <sub>2</sub>	M2670	Sigma
Formaldehyde 37%	553998	Sigma

## Preparing Solutions for Optional Treatment

### RNase A 100X

1. Prepare stock solution of RNase 10mg/mL in 2XSSC.
2. Boil for 15 minutes, aliquot and keep at -20°C for 1 year.

### Pepsin 10% stock solution (100mg/mL)

3. Mix 500mg of pepsin in 5mL of water. Aliquot in 10 $\mu$ L and store at -20°C for 1 year.

### 0.01M HCl

4. Add 50 $\mu$ L HCl 37% (10N) in 50 mL distilled water. Keep at room temperature for 6 months.

### 1M MgCl<sub>2</sub>

5. Mix 101.6g of MgCl<sub>2</sub> hexahydrate in 500mL of water. Keep at room temperature for 6 months.

### **1XPBS 50mM MgCl<sub>2</sub>:**

6. To prepare 1L of solution, mix together 50 mL 1M MgCl<sub>2</sub>, 100mL of 10XPBS and 850 mL water. Keep at room temperature for 6 months.

### **1% Formaldehyde**

7. Add 1.4 mL of 37% Formaldehyde to 48.6 mL of 1XPBS/MgCl<sub>2</sub>. Always prepare this solution directly before use and discard any excess.

### **Optional Treatment Procedure**

1. In a micro centrifuge tube, add 1mL of 2xSSC and 10 $\mu$ L of 100x RNase solution to obtain an RNase solution at 100 $\mu$ g/mL.
2. Add 50 $\mu$ L of RNase solution (100 $\mu$ g/mL) in 2xSSC on each slide and cover with a 22mm x 50 mm glass cover-slip. Incubate the slides for 30 minutes at 37<sup>o</sup> in a moist chamber.
3. Wash the slides 5 minutes in 2xSSC.
4. Prepare the pepsin treatment in a coplin jar by adding 50 mL 0.01M HCl and 10 $\mu$ L pepsin 10% and heat in a 37<sup>o</sup>C water bath.
5. Incubate the slides in pepsin at 37<sup>o</sup>C for 5 minutes.
6. Wash slides in 1xPBS bath for 5 minutes.
7. Wash slides in 1xPBS/MgCl<sub>2</sub> 50mM for 5 minutes.
8. Place the slides in a coplin jar containing 1% formaldehyde, 1xPBS and 50mM MgCl<sub>2</sub> and incubate 10 minutes.
9. Wash slides in 1xPBS for 5 minutes.
10. Dehydrate slides in: 70%, 80%, 90% and 100% ethanol, 2 minutes each.
11. Air dry the slides and proceed with the denaturation step.



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