



## FITC Amplification Protocol (1084-KF)

Approx time: Preparation 20 min.  
 Procedure 70 min.

Equipment	Reagents
Coverslips	NaCl,
Coplin jars	Na citrate
Humidified chamber	HCl
Micro-pipette 1µl, 10µl, 500µl	Double Distilled water
Pipette 10ml, 20ml	Clear nail varnish
Vortex	Formamide
Parafilm	
Micro-centrifuge	
45°C Water bath	
37°C Incubator	
Fluorescence microscope with a suitable filter set	

<b>Kit components:</b>	Detection reagent	F1
	Detection reagent	F2
	Blocking Protein	BP
	Mountant (Antifade) + DAPI	MD
	Detergent	DT

<b>Solutions to be prepared:</b>	20XSSC
	4XSSC
	Solution 1XSSC:
	Stringency wash solution
	Detergent wash solution
	Working Reagent A
	Working Reagent B
Working Reagent C	

---

**Solution 20XSSC:** 87.6g NaCl  
 44.1g Na citrate  
 up to 500ml Double distilled water  
 Adjust pH to 7.4 using concentrated HCl (before finalising water volume), aliquot and autoclave.

**Solution 4XSSC:** 100ml 20XSSC  
 400ml Double distilled water  
 500ml 4XSSC. Mix well

**Solution 1XSSC:** 25ml 20XSSC  
 475ml Double distilled water  
 500ml 1XSSC. Mix well

**Stringency wash solution:** 50ml Formamide  
 50ml 1XSSC  
 100ml Stringency wash solution. Mix well

**Note:** *Stringency wash solution can be reused up to 5 times but should be discarded after 2 months*

**Detergent wash solution:** 500ml 4XSSC  
 250µl Detergent DT  
 500ml Detergent wash solution. Mix well

**Working Reagent A:** 370µl Blocking Protein BP  
 2130µl Detergent wash solution  
 2500µl Diluted Blocking Protein (15%).

**Working Reagent B:** 6µl Detection reagent F1  
 1244µl Working Reagent A  
 1250µl Diluted Detection reagent F1 (1:200).  
 Incubate in the dark for 5 min. Microcentrifuge at 11.000g, for 5min.

**Working Reagent C:** 12.5µl Detection reagent F2  
 1237.5µl Working Reagent A  
 1250µl Diluted Detection reagent F2 (1:100).  
 Incubate in the dark for 5 min. Microcentrifuge at 11.000g for 5min.

---

**Note:** *All volumes of the working reagents are for ten slides.*



## Procedure:

### Washing

1. Pre-warm solutions to 45°C in water bath at least 30 min before starting:
  - Two Coplin jars of Stringency wash solution (50 ml each)
  - Two Coplin jars of Solution 1XSSC (50 ml each)
  - One Coplin jar of Detergent wash solution (50 ml)

**Note:** *The temperature is important. Check the temperature of the solutions in the Coplin jar and not of the water in the water bath.*

2. Take out the slide from the incubator and leave in Solution 1XSSC for 5 min. Take off rubber cement and replace slide in Solution 1XSSC to remove the coverslip.

**Note:** *Do not allow the slide to dry.*

3. Stringency washes:

Wash slides twice by incubating 5min each time in Stringency wash solution (45°C).  
Wash slides twice by incubating 5min each time in Solution 1XSSC (45°C).  
Incubate slide 3 times for 4min in Detergent wash solution. (45°C).

### Amplification

4. Apply 100µl of Working Reagent B onto the slide and cover with Parafilm immediately.
5. Incubate slide in a humidified chamber for 15-20min at 37°C.
6. Remove Parafilm from the slide and wash 3 times 4min in Detergent wash solution at room temperature by emptying and refilling the Coplin jar.
7. Apply 100µl of Working Reagent C onto the slide and cover with Parafilm immediately.
8. Incubate slide in a humidified chamber for 15-20min at 37°C.
9. Remove Parafilm from the slide and wash 3 times 4 min in Detergent wash solution at room temperature by emptying and refilling the Coplin jar.
10. Drain slide well and mount with 50µl of Reagent MD.
11. Apply glass coverslip and seal with nail varnish. Store slides in the dark at 4°C.

**Note:** *You get almost no air bubbles when Reagent MD is applied on the coverslip and the almost dry (but not dried out!) slide is laid down on the coverslip.*

12. View slides using standard epifluorescence filters for FITC and for counterstain DAPI.