VividFISH™FISH Probe Kit

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I. Products

Name	Cat#	Size	Shipping	Store
VividFISH™ FISH Probes	Varies	100 µL (1x)	Dry ice	-20°C, Stable for at least 1 year
AntiFade w/DAPI I	FP201	250 µL (1x)	Dry ice	

II. Probe Description

The VividFISH™ FISH probes contain fluorophore-labeled DNA and blocking DNA in hybridization solution. **Ready to use.**

III. Materials

i. Laboratory Reagents and Equipments:

Ethanol (100%), 20xSSC, NP-40, Rubber cement, Immersion oil for fluorescence microscopy, 22 x 22 mm and 24 x 24 mm cover slips, adjustable pipettes and tips, Timer, Diamond-tipped scribe, Forceps, Coplin slide jars (50 mL), thermometers, Vortex mixer, Slide box with lid, Microcentrifuge, ThermoCycler, Slide warmer, water bath, circulating water bath (73±1°C), Incubator or Slide Hybridizer, Fluorescence microscope equipped with recommended filters.

ii. Solution Preparations (not included):

Pretreatment Solution：50 mL of 2xSSC, 0.5%NP-40, pH7.0. Store at 4°C.

Denaturation Solution： 50 mL of 70% Formamide, 1xSSC, pH7.0, freshly made.

Washing buffer: 100ml of 0.5xSSC, 0.1%NP-40. Store at 4°C

IV. Pretreatment of slides:

Note: for preparation of cell slides, refer to the protocol on GeneCopoeia's website (http://www.genecopoeia.com/wp-content/uploads/2016/04/Cell-slide-prep_2016.pdf).

- Aged Cell or Chromosome slides.
  (a) Pre-warm a slide jar with 50 mL of **Pretreatment Solution** at 37°C in water bath.
  (b) Incubate the slides in the pre-warmed **Pretreatment solution** at 37°C for 30min.
  (c) Dehydrate in 70%, 90% and 100% ethanol each 1 min, then air dry.
  (d) Place in a slide box at room temperature until the next steps.
- FFPE slides: Follow the Pretreatment kit manual of the manufacturer.
  (The FFPE Pretreatment Kit (FP204) is available from GeneCopoeia Inc.).

V. Probe preparation:

- Warm the **FISH probes** to room temperature, and vortex to mix. Spin the tubes briefly. Gently vortex again to mix.

Note: The **VividFISH™ Probes** included in the kit are ready to use. Dilution of the probes may reduce the FISH signal strength.

VI. Hybridization Procedures:

1. Pre-warm a moisture chamber in a 42°C incubator.
2. Pre-warm a slide jar with 50 mL of **Denaturation Solution** at 73±1°C in a circulating water bath.

3. Incubate the pretreated slides from procedure **IV** in the **Denaturation Solution** at 73±1°C for 5 min.
4. Dehydrate in 70%, 90% and 100% ethanol each 1 min, then air dry.
5. Pick 10 µL/slide of FISH probes from procedure **V** into a tube. Denature at 80°C for 5 min. and then place on ice.
6. Spin the tubes briefly. Gently vortex to mix.
7. Apply 10 µL of the FISH probes to each of the slides from Step 4. Remove any air bubbles.
8. Carefully place a 22 x 22 mm cover slip over the FISH probes solution. Allow the solution to slowly spread and fill under the cover slip.
9. Seal the perimeter of the cover-slip with rubber cement,
10. Place the slides in the pre-warmed moisture chamber in the 42°C Incubator for 16– 24h.
11. Proceed to the **Post hybridization Wash** procedure.

VII. Post hybridization Wash

12. Pre-warm a slide jar with 50 mL of 0.5xSSC+0.1%NP40 at 73±1°C in a circulating water bath.
13. Use a fine forceps to remove the rubber cement seal (avoid disturbing the cover slips).
14. Put the slides in a slide jar with 2xSSC. Gently shake to allow cover slips to float away.
15. Put the slides in the pre-warmed 0.5xSSC+0.1%NP40 at 73±1°C for 5 min. Gently agitate slides during the incubation.
16. Transfer slides to 2xSSC+0.1%NP40 at ambient temperature for 1 min,
17. Rinse with ddH_2O, drain excess liquid and air dry,
18. Load 15 µL of **AntiFade w/ DAPI I**. Remove any air bubbles,
19. Apply a 24 x 24 mm cover slip. Store the slide(s) in the dark for 10-15 min. prior to signal enumeration,
20. Observe under fluorescence microscope.

Note: For long term storage of the slides, seal the perimeter of cover slips with clear nail polish, and store at -20°C in dark.

VIII. Appendix: Fluorescence Microscope Specifications

- **Microscope: F**luorescence microscope with 100 watt mercury lamp.
- **Objectives:** 25X to 100X objectives in conjunction with 10X eyepieces. For enumeration of FISH signals, satisfactory results can be obtained with a 60X, or 100X oil immersion objectives.
- **Filters:** The filters are individually designed for specific fluorochromes and must be chosen accordingly.

Fluorochromes	Excitation	Emission	Compatible Filters
DAPI	345 nm	455 nm	DAPI (Blue)
Green	496 nm	520 nm	FITC (Green)
Orange	562 nm	583 nm	Rhodamine Red (Orange)

GeneCopoeia™ Expressway to Discovery

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