VividFISH™ CEP Kit

GeneCopoeia Inc.
9620 Medical Center Drive, Suite 101
Rockville, MD20850, USA
Tel: +1(301)762-0888
Fax:+1(301)762-3888
Web: www.genecopoeia.com

I. Products

Name	Cat#	Size	Shipping	Store
VividFISH™ CEP	Varies	20 µl (5x)	Dry ice	-20°C, Stable for at least 1 year
Hybridization Sol.	FP200	100µl (1x)	Dry ice	
AntiFade w/DAPI I	FP201	250µl (1x)	Dry ice	

II. Probe Description

The VividFISH™ CEP (Chromosome Enumeration Probes) contain fluorophore-labeled DNA and blocking DNA in **hybridization solution**.

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 Hybridizer, Fluorescence microscope equipped with recommended filters.

ii. Solution Preparations (not included):

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

Denaturation Solution： 50ml of 70% Formamide, 1xSSC, pH7.0, store at -20°C.

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 at GeneCopoeia website (http://www.genecopoeia.com/wp-content/uploads/2016/04/Cell-slide-prep_2016.pdf).

i. Aged Cell or Chromosome slides.
   (a) Pre-warm a slide jar with 50ml 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 next steps,

ii. FFPE slides: Follow the Pretreatment kit manual of the manufactory.
   (The pretreatment kit is available in GeneCopoeia Inc.).

V. Probe preparation:

i. Warm the **FISH probes** and the **Hybridization Sol** to room temperature, and vortex to mix well. Spin the tubes briefly, gently vortex again to mix.

ii. For each slide, dilute 2µl of FISH Probes (5x) to 10µl using **Hybridization Sol**. Put on ice.
   Note: The **Hybridization Sol** included in the kit should be used to dilute the probes. Use other hybridization solutions 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 50ml 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 5min,
4. Dehydrate in 70%, 90% and 100% ethanol each 1 min, then air dry,
5. Denature the prepared FISH probes from the procedure **V** at 80°C for 5min. and then place on ice.
6. Spin the tubes briefly, gently vortex to mix,
7. Apply 10 µl of the FISH probes on 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 slowly spread and fill under the cover slip.
9. Seal the surround 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 50ml 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 float away,
15. Put the slides in the pre-warmed 0.5xSSC+0.1%NP40 at 73±1°C for 5min, gentle agitate slides during the incubation.
16. Transfer slides to 2xSSC+0.1%NP40 at ambient temperature for 1min,
17. Rinse with ddH_2O, drain excess liquids 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 10-15min prior to signal enumeration,
20. Observe under fluorescence microscope.

Note: For long time storage of the slides, seal the surrounds of cover slips with nail enamel, 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

GeneCopoeia Inc. , 9620 Medical Center Drive, Suite 101, Rockville, MD20850, USA
Tel: +1(301)762-0888;Fax:+1(301)762-3888;Web: www.genecopoeia.com