

VividFISH™ CEP Kit 2.0

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 List

Name	Cat#	Size	Shipping	Store
FISH CEP probe for human chromosome 1-green	FP001	10 hybridizations	Dry ice	-20°C, Stable for at least 1 year
FISH CEP probe for human chromosome 1-orange	FP002	10 hybridizations		
FISH CEP probe for human chromosome 2-green	FP006	10 hybridizations		
FISH CEP probe for human chromosome 2-orange	FP007	10 hybridizations		
FISH CEP probe for human chromosome 3-green	FP011	10 hybridizations		
FISH CEP probe for human chromosome 3-orange	FP012	10 hybridizations		
FISH CEP probe for human chromosome 4-green	FP016	10 hybridizations		
FISH CEP probe for human chromosome 4-orange	FP017	10 hybridizations		
FISH CEP probe for human chromosome 5-green	FP021	10 hybridizations		
FISH CEP probe for human chromosome 5-orange	FP022	10 hybridizations		
FISH CEP probe for human chromosome 6-green	FP025	10 hybridizations		
FISH CEP probe for human chromosome 6-orange	FP026	10 hybridizations		
FISH CEP probe for human chromosome 7-green	FP031	10 hybridizations		
FISH CEP probe for human chromosome 7-orange	FP032	10 hybridizations		
FISH CEP probe for human chromosome 8-orange	FP037	10 hybridizations		
FISH CEP probe for human chromosome 9-green	FP041	10 hybridizations		
FISH CEP probe for human chromosome 9-orange	FP042	10 hybridizations		
FISH CEP probe for human chromosome 10-green	FP046	10 hybridizations		
FISH CEP probe for human chromosome 10-orange	FP047	10 hybridizations		
FISH CEP probe for human chromosome 11-green	FP051	10 hybridizations		
FISH CEP probe for human chromosome 11-orange	FP052	10 hybridizations		
FISH CEP probe for human chromosome 12-green	FP056	10 hybridizations		
FISH CEP probe for human chromosome 12-orange	FP057	10 hybridizations		
FISH CEP probe for human chromosome 13-green	FP061	10 hybridizations		
FISH CEP probe for human chromosome 13-orange	FP062	10 hybridizations		
FISH CEP probe for human chromosome 14-green	FP066	10 hybridizations		
FISH CEP probe for human chromosome 14-orange	FP067	10 hybridizations		
FISH CEP probe for human chromosome 15-green	FP071	10 hybridizations		
FISH CEP probe for human chromosome 15-orange	FP072	10 hybridizations		
FISH CEP probe for human chromosome 16-green	FP076	10 hybridizations		
FISH CEP probe for human chromosome 16-orange	FP077	10 hybridizations		
FISH CEP probe for human chromosome 17-green	FP081	10 hybridizations		
FISH CEP probe for human chromosome 17-orange	FP082	10 hybridizations		
FISH CEP probe for human chromosome 18-green	FP086	10 hybridizations		
FISH CEP probe for human chromosome 18-orange	FP087	10 hybridizations		
FISH CEP probe for human chromosome 19-green	FP091	10 hybridizations		
FISH CEP probe for human chromosome 19-orange	FP092	10 hybridizations		
FISH CEP probe for human chromosome 20-green	FP096	10 hybridizations		
FISH CEP probe for human chromosome 20-orange	FP097	10 hybridizations		
FISH CEP probe for human chromosome 21-green	FP101	10 hybridizations		
FISH CEP probe for human chromosome 21-orange	FP102	10 hybridizations		
FISH CEP probe for human chromosome 22-green	FP106	10 hybridizations		
FISH CEP probe for human chromosome 22-orange	FP107	10 hybridizations		
FISH CEP probe for human chromosome X-green	FP111	10 hybridizations		
FISH CEP probe for human chromosome X-orange	FP112	10 hybridizations		
FISH CEP probe for human chromosome Y-green	FP116	10 hybridizations		
FISH CEP probe for human chromosome Y-orange	FP117	10 hybridizations		

II. Products Information

Name	Cat#	Size
VividFISH™ CEP	Varies	20 µl (5x)
Hybridization Sol.	FP200	100µl (1x)
AntiFade w/DAPI I	FP201	250µl (1x)

III. Probe Description

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

IV. 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

V. 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.

- Pre-warm a slide jar with 50ml of **Pretreatment Solution** at 37°C in water bath,
- Incubate the slides in the pre-warmed **Pretreatment solution** at 37°C for 30min,
- Dehydrate in 70%, 90% and 100% ethanol each 1 min, then air dry,
- 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.).

VI. 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. .

VII. Hybridization Procedures:

- Pre-warm a moisture chamber in a 42°C Incubator.
- Pre-warm a slide jar with 50ml of **Denaturation Solution** at 73±1°C in a circulating water bath.
- Incubate the pretreated slides from procedure **V** in the **Denaturation Solution** at 73±1°C for 5min.
- Dehydrate in 70%, 90% and 100% ethanol each 1 min, then air dry.
- Denature the prepared FISH probes from the procedure **VI** at 80°C for 5min. and then place on ice.
- Spin the tubes briefly, gently vortex to mix,
- Apply 10 µl of the FISH probes on each of the slides from Step 4, remove any air bubbles.
- 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.

VIII. 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 ddH₂O, 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.

IX. Appendix: Fluorescence Microscope Specifications

- **Microscope:** Fluorescence 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	552 nm	576 nm	5-TAMRA(Orange)

CEP-20260228