

## ReadiLink™ iFluor® 488 FISH Fluorescence Imaging Kit

Catalog number: 17310  
Unit size: 25 reactions

Component	Storage	Amount (Cat No. 17310)
Component A: iFluor® 488-dUTP	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: dNTP mix	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: FISH Reaction mix (2X)	Freeze (< -15 °C), Minimize light exposure	1 vial

### OVERVIEW

Fluorescence in situ hybridization (FISH) technology is an effective tool for detecting specific nucleic acid targets in a biological specimen. Detection of a nucleic acid target in situ is achieved through the hybridization of a fluorescent dye-labeled nucleic acid probe of complementary sequence to the specimen. The ReadiLink™ iFluor® 488 FISH fluorescence imaging kit is a convenient tool for labeling a target DNA using an iFluor® 488 labeled FISH probe via in situ hybridization. The kit provides Taq DNA polymerase enzyme, which incorporates iFluor® 488-dUTPs in the target DNA through Polymerase Chain Reaction (PCR). Our proprietary iFluor® dyes are brighter and more photostable than traditional fluorescent labels, providing the desired resolution and signal.

### KEY PARAMETERS

#### Thermal Cycler

Recommended plate                      PCR Microplate

### SAMPLE EXPERIMENTAL PROTOCOL

Before using, thaw all components to room temperature and mix thoroughly by vortexing.

**Note:** The following protocol can be used as a general guideline to standard DNA FISH. Optimization may be necessary for your experimental system.

1. Prepare the following reaction mixes as indicated in Table 1.

**Table 1.** Reagents composition per well for each reaction.

Components	Volume (25 µL/reaction)	Final Conc.
FISH Reaction mix (2X)	12.5 µL	1X
Upstream primer, 10 µM	0.25-2.5 µL	0.1-1.0 µM
Downstream primer, 10 µM	0.25-2.5 µL	0.1-1.0 µM
DNA template	1-5 µL	Optimized conc.
iFluor® 488-dUTP	2.5 µL	
dNTP mix	1 µL	
Water, nuclease-free	25 µL	

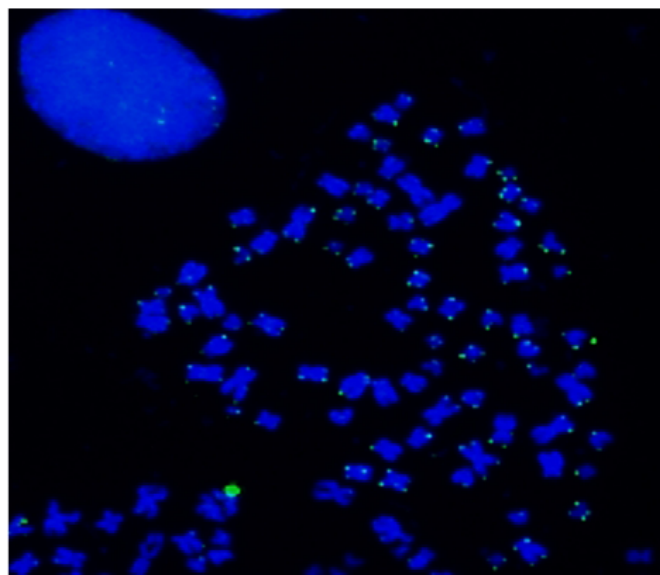
2. Carefully mix the reagents by gentle vortexing followed by a brief centrifuge.
3. Set up the plate in the qPCR instrument and run as indicated in

Table 2.

**Table 2.** Thermal cycling parameters.

Parameter	Polymerase Activation	PCR (30-40 cycles)		
	Hold	Denature	Anneal	Extend
Temperature	95 °C	95 °C	55-65 °C	68-72 °C
Time (m:ss)	0:20	0:30	1:00	1:00

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Telomere quantitative fluorescence in situ hybridization in metaphase HeLa cells using iFluor® 488-dUTP labeled telomere probes. Probes were created using the ReadiLink™ iFluor® 488 FISH Fluorescence Imaging kit.

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