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

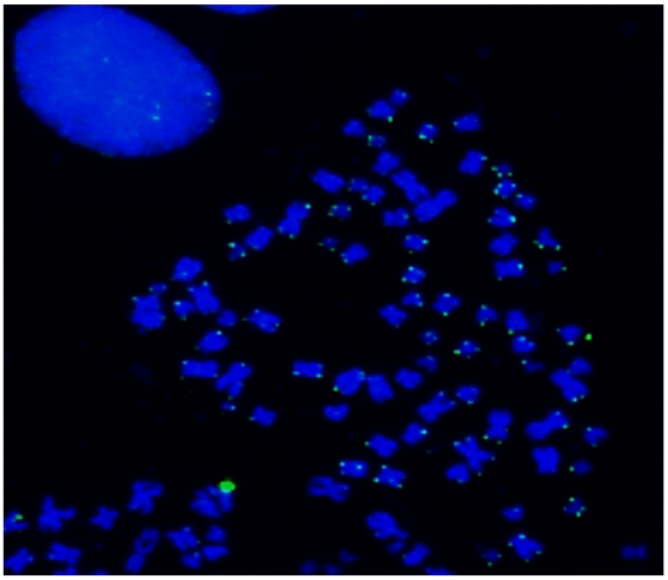

Figure 1. Telomere quantitative fluorescence in situ hybridization in metaphase HeLa cells using iFluor® 488-dUTP labeled telomere probes. Probes were created using the ReadiLink™ iFluor® 488 FISH Fluorescence Imaging kit.

DISCLAIMER

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