Product Information

YF® Dye dUTP Conjugates

Product Size: 25 nmol

Catalog Number:

Catalog Number	Product Name	Mwt	Ex/Em(nm)
YD0045	YF®488(6)-P_4-dUTP	1366.9	490/515
YD0046	YF®555-dUTP	1551.4	555/565
YD0044	YF®594-P_4-dUTP	1547.3	590/617

Storage

Store at -20°C and protect from light. The period of validity is shown in the outer packing. It can be dissolved in 10 mM tris(pH 7.4) and stored in separate packs to avoid repeated freezing and thawing. Expiration date marked on the outer packing.

Protocol

DNA labeling

1. Reagents (Required materials not provided)

(1) Taq DNA polymerase

(2) 10× Taq reaction buffer

(3) 25 mM $MgCl_2$

(4) dATP, dTTP, dCTP, dGTP (separate solution), 1 mM each

(5) DNA template

(6) Forward and reverse primers, 10 μM each

(7) PCR cleaning kit

2. PCR reaction

(1) Prepare the PCR reaction mixture according to the reaction system in Table 1.

(2) Add 1 μL 1 mM YF® dye dUTP dye to each reaction tube

Note: Negative control tube, add 1 μL 1 mM dTTP instead of YF® dye dUTP.

(3) Run the PCR reaction according to the procedure in Table 2.

Note: a. The heat denaturation time is adjusted according to different Taq enzymes;

b. Annealing temperature setting: Tm-5°C;

c. The extension time depends on the size of the amplified fragments, generally 200-300 bp fragments can be set to 1 min.

(4) **Optional steps**. A PCR cleaning kit was used to remove unincorporated single nucleotides.

Table 1 PCR reaction system

Component	volume	Final concentration
10× Taq reaction buffer	2 μL	1×
25 mM $MgCl_2$	2 μL	5 mM
1 mM dATP	2 μL	100 μM
1 mM dCTP	2 μL	100 μM
1 mM dGTP	2 μL	100 μM
1 mM dTTP	1 μL	50 μM
10 μM Forward primer	1 μL	500 nM
10 μM Reverse primer	1 μL	500 nM
template	1 ng	50 pg/μL
Taq	1 U	0.05 U/μL
dH_2O	up to 19 μL	

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Table 2 PCR reaction conditions

94℃ 2 min	Hold
94℃ 30 sec	30 cycles
50-60℃ 30 sec	
72℃ 1 min	
72℃ 5 min	Hold

(5) Take 10% of the PCR products for agarose gel electrophoresis (no DNA dye is added to the gel), to detect the efficiency and specificity of the PCR reaction, and observe by UV gel imager or laser gel scanner. Among them, far-infrared dyes (wavelength ≥650 nm) cannot be observed with the naked eye.

Note: Observe the fluorescence of YF® dye before staining the gel to avoid fluorescence quenching with the gel dye in the next step.

(6) Use the post-staining to stain the gel with DNA gel dye, and observe the total PCR product or the PCR amplification product of the negative control group.

TUNEL method to detect cell apoptosis

Note: UE provides a series of YF® Dye TUNEL Assay Kits. The components of the kit include: equilibration buffer, reaction buffer and TdT enzyme.

1. Reagents (Required materials not provided)

(1) PBS, pH 7.4

(2) 4% formaldehyde in PBS

(3) 70% ethanol (optional)

(4) 0.2% Triton™ X-100 in PBS

(5) 0.1% Triton™ X-100 in PBS/5 mg/mL bovine serum albumin (BSA)

(6) 12.5 U/μL terminal deoxyribonucleotide transferase (TdT)

(7) 5×TdT reaction buffer: 1M potassium dimethylarsenate, 125 mM Tris-HCl, 1.25 mg/mL BSA, pH 6.6

(8) 25 mM $CoCl_2$ solution

(9) 100 μM dATP

2. Sample preparation

(1) Preparation of cells or fresh frozen tissue sections

a. Prepare a sample without TdT enzyme as a negative control. (Optional step)

b. Wash the cells or tissue sections twice with PBS.

c. Add 4% formaldehyde to the above cells or tissue sections and incubate at 4°C for 30 min.

d. Resuspend the cells in 70% ethanol and store at -20°C for two weeks. (Optional step)

e. Wash twice with PBS.

f. Add an appropriate amount of 0.2% Triton X-100 in PBS and incubate at room temperature for 30 min.

g. Wash twice with PBS.

(2) Preparation of paraffin tissue section

a. Prepare a sample without TdT enzyme as a negative control (optional).

b. Perform dewaxing or hydration treatment according to standard procedures.

c. Wash twice with PBS.

d. Use 20 μg/mL proteinase K (in PBS) to promote penetration, treat the tissue, and incubate at 37°C for 30 min. According to the tissue type, the incubation temperature and time of proteinase K can be changed accordingly.

e. Wash twice with PBS.

3. Preparation of reaction mixture

(1) Dilute YF® dye dUTP to 10 μM with deionized water.

(2) Prepare 100 μL TUNEL equilibration buffer for each sample, with the following ratio:

20 μL 5× TdT reaction buffer;

20 μL 25 mM $CoCl_2$;

60 μL dH_2O.

(3) Prepare 50 μL TUNEL reaction mixture for each sample, as shown in the following table:

Component	Volume	Final concentration
5 × TdT reaction buffer	10 μL	1 ×
25 mM $CoCl_2$	10 μL	5 mM

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100 μM dATP	2.5 μL	5 μM
10 μM YF® dye dUTP	2.5 μL	0.5 μM
12.5 U/μL TdT	1 μL	12.5 U/reaction
dH_2O	24 μL	
Total volume	50 μL	

4. TUNEL staining

(1) Add 100 μL of equilibration buffer to the sample and incubate for 5 min at room temperature.

Note: For adherent cells or tissue sections, cover the sample with a paraffin coverslip so that the buffer covers the sample evenly.

(2) Remove the equilibration buffer and add another 50 μL reaction buffer.

Note: For adherent cells or tissue sections, cover the sample with a cover glass so that the buffer covers the tissue evenly.

(3) Incubate for 60 min at 37°C in the dark. Tissue sections need to be incubated for 2 h at 37°C in the dark.

Note: a. For cell or tissue sections, the incubation should be carried out in a humid environment;

b. For suspended cells, the incubation should be carried out on a shaker, or during the incubation, gently shake the reaction solution every 15 minutes.

(4) Wash the sample three times with a PBS solution containing 0.1% Triton X-100, 5 mg/mL BSA, each for 5 minutes.

(5) If necessary, the sample can be counter-stained. Use a fluorescence microscope or flow cytometer to observe. The nuclei of TUNEL-labeled cells showed bright fluorescence. In the control group without TdT enzyme, the cells were not labeled with fluorescence.

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