User Guide

Version 1.0 (April 2007)

Product: **FISH*Bright*™ Nucleic Acid Labeling Kits**
Catalog number (s): FLK-001 – FLK-007
Lot number: See label on package
Unit Size: 10 labeling reactions

For the full manual please visit www.kreatech.com

I. Components & Storage - IMPORTANT

Materials Provided	Quantity	Storage Conditions
ULS hapten/dye	20 µl	2-6°C
10 x Labeling solution	100 µl	2-6°C
KREA*pure*™	10 pcs	2-6°C
Cell Hybridization Buffer	1 ml	2-6°C
Paraffin Hybridization Buffer	1 ml	2-6°C
KREA*boost*	100 µl	-20°C*

* KREA*boost* is shipped at 4°C, store at -20°C upon delivery

Reagents and Buffers not Included in Kit
DNase I, Nicking buffer, Nuclease free H_2O, NH_4AC, EtOH

II. Suggested Fragmentation Protocols

- If DNA fragmentation is performed using DNase I, it must be done **before** ULS labeling. If fragmenting DNA by sonication then this may be carried out before or after ULS labeling
- For in situ hybridization the optimal probe fragment size is between 100-300 bp. Probes over 1 kb will generate a spotted background in *in situ* hybridization. Because most PCR generated probes are smaller than 1 kb, these probes can be labeled without fragmentation. Other probes such as plasmids, cosmids, YACs, PACs and BACs must be fragmented **prior** to labeling with ULS.

a. Suggested Fragmentation by Sonication

1. Prepare at least 100 µL of DNA solution in nuclease free water with a minimal DNA concentration of 50 ng/µL
2. Sonicate for 5 cycles of 1 minute each at amplitude 5µm, keep sample on ice. Cool the DNA solution on ice for 1 minute after each cycle. Check our website (www.kreatech.com) for more protocols.
3. Check the fragment length by electrophoresis on a 1% agarose gel
4. Label the DNA according to the labeling protocol

b. Suggested Fragmentation by DNase I Treatment

1. Prepare DNase I stock solution: dissolve 1mg of DNase I (Roche # 104 159, approx. 2000 units/mg) in 1 mL of 5 mM NaAc pH 5.2, 1 mM $CaCl_2$, 50 % glycerol. Make sure buffer is ice cold before adding the lyophilized DNase I. Mix by inversion. Do not vortex. Store stock DNase I solution at -20°C; avoid freeze-thaw cycles
2. Prepare 10 x nicking buffer: 50 mM Tris-HCl pH 7.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$
3. Dilute the DNase I stock 1:5000 in 1x nicking buffer. The buffer solution should be cold before the addition of the enzyme. These dilutions should be prepared immediately before use
4. Setup nicking reaction on ice as follows:
   - 1 µg template DNA
   - 2.5 µL 10 x nicking buffer
   - 3-5 µL diluted DNase I
   - H_2O to 25 µL
5. Incubate at 37°C for 10 minutes. Stop reaction by placing on ice for at least 1 minute and precipitate with ¼ volume of 10M NH_4Ac and 2.5 volumes of 100% EtOH. Resuspend the Nuclease-free water
6. Check the fragment length by electrophoresis on a 1% agarose gel

III. ULS Labeling

For labeling 1µg nucleic acid sample

1. Briefly spin all required reagents to collect contents of tubes
2. Take 1 µg of nucleic acids sample ensure final concentration in labeling reaction is above 50 ng/µL. (If the final concentration of the nucleic acid sample in the labeling mixture is below 50 ng/µL then the optimal degree of modification is not achieved)
3. Add 2µL of ULS label per 1µg nucleic acid sample
4. Add 1/10 volume of 10x Labeling solution
5. Adjust with Nuclease-free Water to final volume and mix by pipetting (see example set-up below)
6. Label sample by incubation for 30 minutes at 85°C
7. Place samples on ice, spin down to collect contents of tube before proceeding with purification using the KREA*pure* columns

Example of ULS labeling of 1 µg nucleic acid sample in 20µL

Nucleic acid sample in Nuclease-free Water	16 µL
ULS dye	2 µL
10 x labeling solution	2 µL
Total volume	20 µL

Dye removal using KREA*pure* columns
(20800 x g is equivalent to 14,000 rpm on eppendorf 5417C)

1. Resuspend column material by vortexing
2. Loosen cap ¼ turn and snap off the bottom closure
3. Place the column in a 2 mL collection tube
4. Pre-spin the column for 1 minute at 20800 x g
5. Discard flow through and re-use collection tube
6. Wash the column with 300 µL nuclease-free water
7. Discard collection tube and flow-through

8. Put column in a new (nuclease free) 1.5 mL micro centrifuge tube
9. Add ULS-labeled nucleic acid on to column bed
10. Spin column for 1 minute at 20800 x g
11. Flow through is purified labeled nucleic acid sample. At this point the degree of labeling (DOL) can be measured

$$X = \text{ng (nucleic acid) / µl} = \frac{OD260 * \text{dilution factor} * 50}{\text{cuvet length (cm)}}$$

$$Y = \text{pmol (dye) / µl} = \frac{ODdye * \text{dilution factor}}{\text{cuvet length (cm)} * \varepsilon \text{ dye} * 1 \times 10^{-6}}$$

$$\textbf{Degree of Labeling (DoL)} = \frac{340 * Y * 100\%}{X * 1000}$$

ε dye ULS-Dy415 = 56 000
ε dye ULS-FLU = 83 000
ε dye ULS-D-Green = 78 000
ε dye ULS-RHO = 95 000
ε dye ULS-Dy547 = 150 000
ε dye ULS-Dy647 = 250 000

IV. Post labeling

Slide Pretreatment:

For use on metaphase and interphase cells from peripheral blood cultures or direct preparations prepared by standard cytogenetic methods, see: The ACT cytogenetics laboratory manual. 2nd ed. New York: Raven Press; 1991.
Pre-treat dry sample slide in 2 x SSC, pH 7.0 at 37°C for 2 minutes. Dehydrate in 70%, 85% and 100% Ethanol for 1 minute each. Air dry at room temperature.

Hybridization recommendations

Probe type	Concentration	Time
PACs/BACs	40 -100 ng	O/N
YACs	100 - 400 ng	O/N to 72 hours
PCR	10 - 40 ng	O/N
Repeats	1-10 ng	2 hrs to O/N

Buffer/component	Use for:
cell hybridization buffer	Inter-metaphases/ blood smears/ cell cultures/
tissue hybridization buffer	High background/ paraffin embedded/
KREA*boost*	Paraffin embedded/ Aspecific

For paraffin embedded tissue sections please refer to the Appendix on page 15 of the FISH*Bright*™ Instruction Manual. For slides with cytoplasmic background or difficult samples refer to page 16 of the same manual. This manual can be downloaded by visiting www.kreatech.com

NOTES:

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