FT-AK218A

5-Aminoallyl-dUTP

Product Information

Name :	**5-Aminoallyl-dUTP, sodium salt (aa-dUTP)**
	5-(3-aminoallyl)-2'-deoxyuridine-5'-triphosphate, trisodium salt
Catalog Number :	FP-AK218A, 100 µl (10 mM in TE buffer)
	FP-AK218B, 250 µl (4 mM in TE buffer)
	FP-AY490A, 1 mg
	FP-AY490B, 1 mg – FluoProbes® Pure Grade
	FP-AY490C, 20 x 50 µg – FluoProbes® Pure Grade
Structure :	$C_{12}H_{17}N_3Na_3O_{14}P_3$
Molecular Weight :	589.17
Soluble in:	Water, DMSO

Storage: –20°C >1 year. (M)

Introduction

5-Aminoallyl-dUTP can be used to produce amine-containing DNA by conventional enzymatic incorporation methods such as reverse transcription, nick translation, random primed labeling, or PCR. Aminoallyl dUTP can be readily incorporated into DNA through the conventional enzymatic incorporation techniques. The resulting amine-modified nucleic acids can then be subsequently labeled using any of amine-reactive fluorescent dyes, biotins and other amine-reactive reagents. The aminoallyl-modified nucleotides can be incorporated to extremely high and consistent levels compared to the tag-labeled uridine triphosphates that generally have higher stereo-hindrance. Subsequent reaction of the amine-modified nucleic acid with an excess of amine-reactive reagent achieves correspondingly high and consistent labeling efficiencies, regardless of the labeling reagent chosen. This two-step labeling method also eliminates the need to optimize an enzymatic reaction to accommodate different dye-modified nucleotides, which may incorporate at very different rates. This labeling method is widely used for both FISH probes and microarray-based experiments.

Protocol

Incorporation of aa-dUTP by Reverse Transcription (Joseph DeRisi, June 2001)
AminoAllyl-UTP (aa-UTP) is incorporated for subsequent labeling by NHS-FluoProbes®dye.

1- Make the dNTP + aa-dUTP mixture:

	Volume (µL)
100mM dATP	10
100mM dCTP	10
100mM dGTP	10
100mM dTTP	6
100mM aa-dUTP	4
total dNTP + aa-dUTP mixture (50x)	*40µL*

2- Set up the Priming Reaction:

	[concentration]	*µL*
Oligo dT / Random Primer	2µg/µL each	1
poly(A)+ RNA	2µg total	14.5
total Priming Reaction per rxn		*15.5µL*

3- Incubate the priming reaction at 70°C for 8 minutes. Remove and put on ice.

4- Set up the cDNA synthesis reaction:

	[concentration]	*µL*
RT Buffer	10x	3
aa-dNTP	50x	0.6
DTT	0.1M	3
Reverse Transcriptase	50U/µL	2
Water		5.9
total cDNA Synthesis Reaction per rxn		*14.5 µL*

5- Add 14.5µL of master mix to each Priming Reaction.
6- Incubate reactions at 42°c for 2 hours.

Hydrolysis and Cleanup

1- Bring cDNA synthesis reactions to a final concentration of 100mM NaOH and 10mM EDTA. Incubate at 65°C for 10 minutes.

2- Neutralize the hydrolysis reaction by the addition of HEPES, pH 7.0, to a final concentration of 500mM. Other non-primary amine containing buffers may also be used. Note that Tris buffer carries a free amine and should be avoided since this could possibly interfere with the subsequent coupling reaction.

3- Bring the reaction volume to 500µL with water. Concentrate the cDNA product by filtering through a Microcon-30. Try to get the final volume of the sample down to below 10µL. This can usually be accomplished by spinning at full speed for 6-10 minutes in a typical microcentrifuge. Do not spin to dryness as this can make the cDNA difficult to recover.

4- Bring the concentrated product to 500µL and repeat the concentration at least twice. The net effect of this process is to remove the hydrolyzed RNA, NaOH, and buffer components.

5- The amino-allyl labeled cDNA may now be stored indefinitely at -20C.

Aliquoting FluoProbes®-dye esters

1- Resuspend the solid pellet in 12µL of water free DMF or DMSO.

2- Since a single tube of dye usually provides sufficient material to label at least 12 samples, aliquot 1µL volumes of the resuspended dye into separate screw cap tubes. Dry down the aliquots using a speed-vac, without heat.

3- Store the dye aliquots at 4C, in a light-sealed box, preferably under vacuum and in the presence of a large amount of desiccant; This will help ensure the dyes remain uncontaminated with moisture.

Coupling to N-hydroxysuccinimidyl ester dyes

1- Bring the cDNA solution to a final volume of 10µL with water. Add XXµL of 1M sodium bicarbonate buffer, pH 9.0.

2- Remove a dye aliquot from storage and use the bicarbonate buffered cDNA solution to vigorously resuspend the pellet by pipetting up and down.

3- Incubate the coupling reaction in the dark for at least 60 minutes at room temperature.

Removal of uncoupled dye material

A PCR Purification columns like UptiDNApure DNA purification kit work well for removal of uncoupled dye. Purify each dye labeled sample separately by following the manufacturers directions with the following modifications:
1- Mix in Buffer B (500µL) with the coupling reaction before application to the DNA binding column.

2- Rinse the column with 600µL of Buffer PE at least two times.

3- After the final rinse, spin the column one more additional time to remove any traces of the rinse buffer.

4- Add 60µL of elution Buffer EB to the column and let incubate for 5 minutes at room temperature. Spin eluate through to a collection tube.

5- Repeat the elution with another 60µL of Buffer EB.

6- Concentrate the eluate to desired volume by vacuum drying or by concentration using a Microcon-30 spin filter.

References

-*Biol Chem Hoppe Seyler*, **371**, 953 (1990)
-*Biol Chem Hoppe Seyler*, **371**, 953 (1990)
-*Biotechniques*, **28**, 518 (2000)
-**Peter A. C. 't Hoen**, *et al*, « Fluorescent labelling of cRNA for microarray applications », *Nucleic Acids Res*., **31**, 20(2003) Article

Related products

- UptiDNApure™ DNA purif. kit from PCR, UPS54324
- MonoFas® DNA purification kit from PCR, BN3310
- dNTP Set 1 (100mM each), UP968640
- SP6 RNA Polymerase 60U/µl, BL9610
- Maxime RT premix oligo-dT(15) primer, CD9240
- Dithiothreitol, UP284250
- FluoProbes® 488-dUTP PCR Grade, FP-CD0610
- FluoProbes® 488-dUTP Nick Translation grade, FP-CI8870

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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