UniScript™ Taq Labeling Kit

Product Number **US-TAQ**
Technical Bulletin No. MB-990

Storage Temperature –20 °C

ProductInformation

TECHNICAL BULLETIN

Product Description

Sigma's UniScript™ Taq Labeling Kit provides a convenient method for producing hapten labeled single-stranded DNA probes from single or double stranded templates. The method is a one-step linear amplification-labeling procedure that uses *Taq* DNA polymerase and a single primer to incorporate hapten-modified dUTPs into a single stranded amplification product. A significant advantage of this method is that sense or anti-sense probes can be prepared from any double stranded template without the need for RNA polymerase promoters.[1] Probe sequence is entirely dictated by the user-designed primer. In addition, single-stranded DNA probes do not require denaturation prior to hybridization and are not susceptible to RNase degradation.[2] Probes constructed with this kit are as sensitive in blotting applications as those generated by random-primed and *in vitro* transcription labeling methods.[3] The kit has been optimized for producing labeled probes less than 1 kb in length utilizing fluorescein-12-dUTP, digoxigenin-11-dUTP or biotin-16-dUTP (labeled deoxyribonucleotides not included).

Components

Sufficient for 50-20 µl reactions

- 10X Taq Labeling Buffer, Product Code B 7805 — 0.1 ml
- 10X Taq Nucleotide Mix, Product Code N 1774 — 0.1 ml
- 20X Taq Polymerase Stock, Product Code D 3064 — 0.05 ml
- Molecular Biology Grade Water, Product Code W 4502 — 1 ml

Reagents Required But Not Provided

- Thermocycler
- Purified and diluted dsDNA template
- Gene-specific oligonucleotide primer
- Fluorescein-12-dUTP (Roche) **or**
- Biotin-16-dUTP (Roche) **or**
- Digoxigenin-11-dUTP (Roche)
- SigmaSpin™ Post-Reaction Purification Columns, Product Code S 5059

Precautions and Disclaimer

Sigma's UniScript Taq Labeling Kit is for R & D use only. Not for drug, household, or other uses.

Storage/Stability

Store all reagents at –20 °C. Components are shipped on dry ice. Under proper storage conditions, kit reagents are stable for 1 year from date of receipt. The molecular biology water grade may be stored at room temperature for convenience.

Procedure

A. Template DNA Preparation

The template for the labeling reaction is a double or single-stranded DNA template of defined length, typically a PCR† product. To obtain optimum probe synthesis, it is recommended that the template be essentially free of contaminating PCR primers, nucleotides and buffer salts. PCR product purification can be effected using silica based bind and elute methods such as with the GenElute™ PCR DNA Purification Kit (Product Code GEN-PCR). After the template concentration is determined by standard spectrophotometric or fluorometric methods[4-6], it should be diluted to approximately 0.1 pmoles/µl. The target concentration of length (L) template, in µg/ml, can be conveniently calculated using 0.066 x L and 0.033 x L for double stranded templates and single stranded templates respectively. Alternatively, template can be diluted according to the following equations, ds and ss for duplex and single stranded templates respectively.

$$V_{water} = \frac{T}{0.033 \times L} - 2 \qquad \text{eq. ds}$$

$$V_{water} = \frac{T}{0.0165 \times L} - 2 \qquad \text{eq. ss}$$

T is concentration of template in µg/ml.

L is the template length.
V_{water} is the volume of water (µl) to which 2 µl of template is added.

B. Labeling Reaction

The following procedure is for a 20 µl reaction. The reaction can be scaled to 50 µl without affecting performance.

1. Completely thaw all reagents and mix prior to use. For maximum shelf life keep the reagents on ice when not in use.
2. Combine the following to a final volume of 20 µl and mix well.

Reagent	Volume
10X Taq Labeling Buffer	2 µl
10X Taq Nucleotide Mix	2 µl
20X Taq Polymerase Stock	1 µl
1 mM hapten-labeled dUTP	0.5 µl
DNA template (diluted as described in Section A or 0.2 pmoles)	2 µl
Oligonucleotide primer	60 pmol
Molecular biology grade water	q.s. 20 µl
Total volume	20 µl

3. Thermocycling
   Listed below are recommended cycling parameters:

Denaturation	95°C	2 min	
Denaturation	95°C	15 sec	
Annealing*	T°C	30 sec	30 cycles
Extension	72°C	90 sec	
Final Annealing	72°C	5 min	

*The optimal annealing temperature can be estimated from the number of GC bases contained in the oligonucleotide primer (see table below).

# GC Bases	Typical Annealing Temperature (T °C)
7-8	45 °C to 50 °C
9-10	50 °C to 55 °C
11-12	55 °C to 60 °C
13-14	60 °C to 70 °C

C. Purification

1. The labeled probe can be purified from unincorporated nucleotides and buffer components by size exclusion chromatography using SigmaSpin Post-Reaction Purification Columns (Product Code S 5059) or similar methods.
2. Probe synthesis can be confirmed by agarose gel electrophoresis of 1 to 3 µl of probe. If fluorescein-12-dUTP is incorporated, the probe can be directly visualized in the gel on a UV transilluminator, otherwise, the probe can be observed by staining the gel with ethidium bromide.

D. Use

The probe can be used in standard blotting/ hybridization protocols ignoring probe denaturation steps at approximately 0.1 to 1 nM (i.e. approximately 1:10,000 to 1:1000 for an efficient amplification).

Troubleshooting Guide

Problem	**Cause**	**Solution**
Low or no probe production	Annealing temperature too high	Reduce the annealing temperature 5-10 °C and repeat.
	Inadequate amount of DNA	Verify correct DNA concentrations and calculations.
	Incorrect primer	Repeat the labeling reaction with correct primer.
Multiple probe bands observed	Multiple template bands	Gel or other template purification
	Annealing temperature too low	Increase the annealing temperature in 5 °C increments and repeat. Reduce annealing temperature as necessary to achieve desired probe.
Weak or no detection signal in blotting	Incorrect probe strand synthesized	Repeat the labeling reaction with correct primer.
	Not enough probe in hybridization mix	Add more probe to the hybridization reaction.
	Not enough target present in blot	Repeat experiment with more target and re-probe.

Related Products

Product Name	Product Code.
UniScript™ Transcription Kits with T3 RNA Polymerase, T7 RNA Polymerase or SP6 RNA Polymerase	US-T3, US-T7, US-SP6
BioBond™ Nylon Membrane	N 3781, N 1281
GenElute™ PCR DNA Purification Kit	GEN-PCR
PerfectHyb Plus™ Hybridization Buffer	H 7033
CDP-*Star*™ Universal Detection Kit	U-ALK

References

1. Peterhaensel, C., *et al*., Nonradioactive Northern blot analysis of plant RNA and the application of different haptens for reprobing. Anal. Biochem., **264**, 279-283 (1998).
2. Konat G.W., Generation of high efficiency ssDNA hybridization probes by linear polymerase chain reaction (LPCR). Scanning Microsc Suppl, **10**, 57-60 (1996).
3. Martin, R., *et al.*, A highly sensitive, nonradioactive DNA labeling and detection system. Biotechniques*,* **9**, 762-768 (1990).
4. Sambrook, J., *et al*., *Molecular Cloning: A Laboratory Manual,* 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989) pg. E5
5. Romppanen, E.L., *et al*., Optimal use of the fluorescent PicoGreen dye for quantitative analysis of amplified polymerase chain reaction products on microplate. Anal. Biochem., **279**, 111-114 (2000).
6. Mansfield, E.S., *et al*., Nucleic acid detection using non-radioactive labelling methods. Mol. Cell Probes, **9**, 145-156 (1995).

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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