Nick Translation

A-1

Effective Removal (>99.99%) of free Nucleotides from Nick Translation Reactions using Centri•Spin™-20

Introduction:

Nucleic acid hybridization with labeled probe sequences is a well known analytical tool used in molecular and cell biology. The utility of hybridization has been greatly increased by the development of in vitro methods for incorporation of label (either isotopic or nonisotopic) into the nucleic acid probe molecules. One of these methods is nick translation, where nucleotides are incorporated into double stranded DNA by DNA Polymerase 1 at sites where the molecule has been nicked by DNAse 1. Nucleotide mixes containing a label (32P-dATP or Fluorescein-dUTP) provide the pool of nucleotides which DNA Polymerase 1 will incorporate into the double stranded DNA molecule. The excess labeled nucleotides are then separated from the finished, "labeled" DNA.

The materials to carry out these reactions are available in kit form from many suppliers. Typically the protocols recommend ethanol precipitation to separate the unincorporated nucleotides from the DNA. A volume of a high salt solution (e.g. 7.5 - 10 M ammonium acetate) is added followed by 2-2.5 volumes of ethanol with an extended high-speed centrifugation for an average of 30 min. The pellet is hydrated and the precipitation is repeated. A double precipitation is often recommended to get an effective (>99%)removal of nucleotide label. This process takes time (35-45 min./precipitation) and can result in losses (>30%) within each precipitation.

Centri·Spin-20 *columns are an excellent alternative to lengthy ethanol precipitation steps and result in >99.99% removal of excess label with an 85% or greater yield of labeled DNA*.

Method:

To demonstrate the effectiveness of Centri·Spin-20 for this application, a nick translation experiment was conducted using a commercially available kit, (Nick Translation System, Life Technologies) following the recommended protocol. A nucleotide mix consisting of 20 mM each dATP, dCTP, dGTP and a mix of 15 mM dTTP + 5 mM fluorescein 12-dUTP (Stratagene) was used to label 1 ug pBR322. DNA polymerase 1 (2.5 IU) and DNAse 1 (2.0 IU) were added and the labeling reaction carried out at 15ºC for 60 min. (total volume = 50-100 mL). To stop the reaction, a 1/10 volume of 0.5 M EDTA (pH 8.0) was added. An analogous control reaction contained all the above components except the pBR322 template. A 20 or 50 mL aliquot of each labeling reaction was cleaned using a Centri-Spin 20 column (following the standard protocol) or by ethanol

To Order call 800.223.0902
Fax 732.431.3768
e-mail: info@prinsep.com
internet: http://www.prinsep.com

precipitation as follows: 0.5 volume of 7.5 M ammonium acetate was added to the reaction and mixed followed by 2 volumes of ethanol, mixed, then centrifuged at 12,000 x g for 30 min. The pellet was resuspended in the starting volume of TE (pH 8.0) and the protocol repeated. The fluorescence intensity of each sample was measured compared to the original labeling reaction mix (see Table 1).

TABLE 1- Removal of unincorporated label from 50 mL reaction.

Step	Fluorescence Intensity	Yield (%)
Reaction 1 (pBR322)	16,800	100
Centri·Spin-20	540	3.2
Ethanol precipitation	126	0.75
Reaction 2 (control[1])	17,550	100
Centri·Spin-20	0.95	0.005
Ethanol precipitation	3.68	0.021

[1]. Control reactions contained no template pBR322, only labeled nucleotides

Conclusion:

Centri•Spin-20 (with 50 mL reaction volumes) removed >99.99% of excess label with a nearly quantitative recovery of labeled DNA. The total time for this operation was about 6 minutes. Although double ethanol precipitation removed 99.98% of excess label, only 23 % of the original labeled DNA was recovered relative to the recovery with **Centri•Spin-20**, and the total time for the operation was 75 minutes. Ethanol precipitation gave equivalent results with either 20 or 50 mL sample volumes (data not shown). Reducing the loading volume onto the **Centri•Spin** from 50mL to 20 mL resulted in nearly complete removal of excess label (>99.999%) with equivalent yield of labeled DNA as compared to a 50 mL sample.

Centri-Spin 20 columns provided rapid, effective cleanup of labeled DNA, superior in performance compared with ethanol precipitation for the removal of excess label following nick translation.