Amplicon Rx™

Technical Information Sheet

Cat# 6000

Introduction

Amplicon Rx™ has been designed specifically for post-PCR recovery of STR amplicons from forensic DNA multiplex PCR reactions. The kit will preferentially bind and elute PCR amplicons from a mixture of amplified DNA and unincorporated oligonucleotide primers.

The kit uses a proprietary silica-based resin and non-chaotropic buffer to bind amplified DNA fragments to the membrane of the spin column while unincorporated PCR primers, nucleotides and salts are washed through. No additional washing steps are required or recommended. The ratio of oligonucleotides and buffer is important for the proper functioning of the resin. The method conveniently elutes the PCR-generated DNA fragments into formamide LIZ/ROX, which can then be loaded directly on a capillary electrophoresis instrument for analysis (dye-labeled DNA size standards do not interfere with the elution of amplicons).

The kit will concentrate and purify the PCR-generated amplicons such that the relative fluorescent units (RFUs) of the sample can be increased by at least 5-6 fold. A variety of different samples can be processed with Amplicon Rx™ including 'touch DNA', low template DNA, low copy number samples, or any multiplex DNA-STR reaction where RFU yields are considered less than optimal.

Total hands-on time is approximately 10 minutes.

Kit Components (20 purifications)

Binding Buffer – 3 mL
Spin Columns – 20
Collection Tubes – 40

User-Supplied Materials

Micro Centrifuge
CE quality formamide
LIZ/ROX size standards
General mol bio laboratory materials

Kit Storage Conditions

The Amplicon Rx™ kit is stable for at least 12 months at room temperature (14 – 25°C).

Amplicon Rx™ Post-PCR Purification Procedure

1) Place spin column in the collection tube

2) Add Binding Buffer to the PCR reaction – use a ratio of 5:1 (buffer to PCR reaction)

- e.g., from a full size multiplex PCR reaction (assumes 1.5 µL was removed for initial analysis) 23.5 µL + 117.5 µL Binding Buffer
- e.g., from a half-size multiplex PCR reaction (assumes 1.5 µL was removed for initial analysis) 11 µL + 55 µL Binding Buffer

3) Vortex to thoroughly mix PCR reaction and Binding Buffer

4) Load mixture onto spin column

5) Centrifuge at 12,000 x g for 3 minutes (do not reduce spin time)

6) Discard collection tube and run-through liquid

7) Place spin column in a fresh collection tube

8) Add 25 µL of modified ratio formamide + LIZ/ROX mixture to the spin column:

- e.g., for three samples (amplification negative control + extraction blank + questioned sample) mix 80 µL of formamide with 0.4 µL of LIZ
- e.g., for three samples (amplification negative control + extraction blank + questioned sample) mix 80 µL of formamide with 1.0 µL of ROX

9) Incubate spin column for 5 min at room temperature (do not reduce incubation time)

10) Centrifuge at 12,000 x g for 2 minutes (do not reduce spin time)

11) Sample is now ready for capillary electrophoresis

***Notes and Recommendations:** The reduced volume of dye-labeled size standards has been chosen to maximize amplicon electrokinetic injection. We recommend 5 second injection times for Amplicon Rx™ processed samples. The increase in RFU signal may vary with the sample, the multiplex STR kit used, the CE instrument and run conditions.

Not for *in vitro* diagnostic use

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