

Instructions for Use of E1A&SV40LTA Residual DNA Detection Kit (Multiplex qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- EA001

Introduction

The E1A&SV40LTA residual DNA detection kit can rapidly and specifically detect E1A and SV40LTA DNA residues derived from host cells (such as HEK293T cells) in biological products.

This kit is based on the principle of fluorescence probe and uses multiplex qPCR method to achieve rapid detection with potent specificity, and the lowest limit of detection (LLOD) can be up to 40 copies/ μ L.

This kit is provided with E1A&SV40LTA quantitative reference, and the detection range is: 4×10^6 copies/ μ L ~ 4×10^1 copies/ μ L.

Formula: Plasmid copy number (copies/ μ L) = $6.02 \times 10^{14} \times$ plasmid concentration (ng/ μ L)/(plasmid base number \times 660).

Specification

100 Reactions

Kit components

Table 1: Kit components and storage conditions

Components	Specification	Storage conditions
E1A&SV40LTA Primer&Probe MIX	550 μ L \times 1 vial	-20 $^{\circ}$ C or below, protected from light
2 \times qPCR Reaction MIX	1.6 mL \times 1 vial	Store at or below -20 $^{\circ}$ C
Reference (copies/ μ L)		
Quantitative Reference 1 (4×10^6)	300 μ L \times 1 vial	
Quantitative Reference 2 (4×10^5)	300 μ L \times 1 vial	
Quantitative Reference 3 (4×10^4)	300 μ L \times 1 vial	
Quantitative Reference 4 (4×10^3)	300 μ L \times 1 vial	
Quantitative Reference 5 (4×10^2)	300 μ L \times 1 vial	
Quantitative Reference 6 (4×10^1)	300 μ L \times 1 vial	
DNA diluent	1 mL \times 3 vials	

Shelf life

The shelf life is 12 months when stored at specified storage conditions.

Apparatus to be prepared by the user

Fluorescent quantitative PCR system	Pipettes of 1000 μL, 100 μL, and 10 μL specifications
1.5 mL sterile centrifuge tube	Sterile low-attachment pipette tips with cartridge, 1000 μL, 100 μL, and 10 μL specifications
Sterile, enzyme-free 8-strip PCR tubes or 96-well qPCR plate	

Applicable models (including but not limited to)

- ◆ ABI QuantStudio 3 qPCR System
- ◆ ABI QuantStudio 5 qPCR System
- ◆ Roche LC96 Real-Time PCR System
- ◆ ABI 7500 Real-Time PCR System
- ◆ Bio-Rad CFX Opus96 Real-Time PCR System

Test procedures

Preparation and addition of qPCR reaction solution

1. Calculate the Number of reaction wells required for the reaction according to the number of references to be tested and test sample (generally, 3 sets of duplicate wells are used):

Number of reaction wells = (5 or 6 sets of references + 1 no-template control (NTC) + test sample) × 3

2. Calculate the total amount of E1A&SV40LTA qPCR MIX required for this time based on the number of reaction wells:

E1A&SV40LTA qPCR MIX = (number of reaction wells + 2 or 3) × 20 μL (2 or 3 is the amount of operational loss)

3. Thaw the reagents on ice, shake gently to mix well, and prepare the E1A&SV40LTA qPCR MIX as shown in Table 2.

Table 2 E1A&SV40LTA qPCR MIX Preparation Table

Components	Volume required for single reaction
2×qPCR Reaction MIX	15 μL
E1A&SV40LTA Primer&Probe MIX	5 μL
Total volume	20 μL

4. Place each reagent on ice to thaw, gently shake to mix well, and load to wells according to Table 3 (total volume: 30 μL).

Table 3. Examples of loading to each reaction well

Standards	10 μL each of quantitative references 1 to 6 + 20 μL of required qPCR MIX
No template control	10 μL each of DNA dilution + 20 μL of required qPCR MIX

(NTC)	
Test sample	10 μ L each of test sample + 20 μ L of required qPCR MIX

- The analysis can be carried out using sterile and nuclease-free 8-strip PCR tubes or 96-well plates. Bubbles must be removed from the reaction system. Centrifuge to allow the liquid to gather at tube bottom for reaction.

qPCR reaction program and parameter setting

Taking the CFX96 qPCR system (BIO-RAD) as an example.

- Set up the reaction program:
- Create the experimental reaction plate, click Select Fluorophores to select fluorescence FAM and VIC; in the reaction plate diagram, select the Sample well, pull down in Sample Type to select Unknow, check the fluorescence FAM, Target Name is designated as E1A-DNA; check the fluorescence VIC, Target Name is designated as SV40LTA-DNA, input the number of replicates per sample and Sample Name.

In the reaction plate diagram, select the Standard Curve well, pull down in Sample Type to select Standard, check the fluorescence FAM, Target Name is designated as E1A-DNA; check the fluorescence VIC, Target Name is designated as SV40LTA-DNA, and input the number of replicates for each dilution gradient and Sample Name. And the Concentrations column of STD1, STD2, STD3, STD4, STD5 and STD6 is assigned with values of 4E + 06, 4E + 05, 4E + 04, 4E + 03, 4E + 02 and 4E + 01 (in copies/ μ L), respectively.

- Click "Start Run" on the "Run" interface to perform PCR analysis.

Stage1	Contamination digestion	Reps: 1	50 $^{\circ}$ C	2min
Stage2	Pre-denaturation	Reps: 1	95 $^{\circ}$ C	20s
Stage3	Cyclic reaction	Reps: 40	95 $^{\circ}$ C	3s
			60 $^{\circ}$ C	30s

Set the collection of fluorescence in Stage 3 of the program (at 60 $^{\circ}$ C for 30 seconds);

Reaction volume is 30 μ L

qPCR result analysis

Taking the CFX96 qPCR system (BIO-RAD) as an example.

- Click "Quantitation" on the data analysis window to read the slope, intercept, amplification effect, and R² of the standard curve.
- In the "Quantitation Data" window, the RCL detection values (in copies/ μ L) of NTC and test samples can be read in the "SQ Mean" column.
- For NTC, the result should be N/A, or the Ct value should be greater than the mean Ct value of the lowest concentration on standard curve.

Test procedures

1. This kit has passed the verification of stability (freeze-thaw, tube material and other factors) and no aliquots are required.
2. The environment for the preparation of negative samples (2 × qPCR Reaction MIX, plasmid Primer&ProbeMIX and DNA diluent, etc.) and positive samples (reference and test samples, etc.) should be performed in different areas and should not be operated in a same area. The personnel performing the preparation should wear properly, wear masks, gloves and cleanroom clothing.
3. It is important to carry out timely replacement of the tip between different sample addition steps to avoid cross-contamination and avoid prolonged opening of the cap.
4. The kit must be used within the shelf life; all components in the kit are recommended to be used after thawing at low temperature.
5. The optimal assay results may only be achieved by strictly following the instructions and using only the reagents provided in the kit.
6. The final assay results are closely related to reagent effectiveness, the operations of analysts, and the test environment.
7. The company is only responsible for the kit itself, but not for the sample consumption caused by the use of the kit. The user should take full account of the possible use of the sample before use.
8. This kit is for *in vitro* detection only, and may not be used for clinical diagnosis.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 FAX: 617-419-1110

www.EagleBio.com info@eaglebio.com



EAGLE
BIOSCIENCES

