

RCA (E1A) Copynumber Detection Kit

Product Description

The RCA (E1A) Copynumber Detection Kit can be used to quantitatively analyze and detect the potential risk of Replication-competent Adenoviruses (RCA) that may occur in a variety of cellular therapeutic products and gene therapy products associated with the use of adenoviral vectors; and can also be used to quantitatively analyze and detect the presence of residual DNA of the risk gene E1A in biologics from host cells, such as HEK293 and HEK293T cell-derived risk gene E1A residual DNA.

This kit designs specific primers for the E1A gene sequence on adenovirus, and adopts the principle of fluorescence quantitative PCR by probe method to rapidly and specifically detect the risk of replicative adenovirus RCA and the residual DNA of E1A from the host cell, and the limit of quantification can be as low as 4.1 copies/ μ L, and it contains the RCA (E1A) DNA Control (DNA quantitative reference product). The kit needs to be used together with the the Residual DNA Sample Preparation Kit (Cat# 18461ES).

Specifications

Cat.No.	41321ES50 / 41321ES60
Size	50 T / 100 T

Components

Components No.	Name	41321ES50	41321ES60
41321-A	RCA (E1A) qPCR Mix	0.75 mL	1.5 mL
41321-B	RCA (E1A) Primer&Probe Mix	200 μ L	400 μ L
41321-C	DNA Dilution Buffer	1.8 mL $\times$ 2	1.8 mL $\times$ 4
41321-D	RCA (E1A) DNA Control (1.64×10^7 copies/ μ L)	25 μ L	50 μ L
41321-E	IC [*]	50 μ L	100 μ L

^{*}IC: Internal control.

Storage

This product should be stored at -25~-15°C for 2 years.

Both 41321-A and 41321-B should be stored protected from light.

Applicable instrument models

Include but not limited to:

Bio-Rad: CFX96 Optic Module.

Thermo Scientific: ABI 7500; ABI Quant Studio 5.

Instructions

1. RCA (E1A) DNA Control dilution and Standard curve preparation

The RCA (E1A) DNA Control was gradient diluted using the DNA Dilution Buffer provided in the kit^{*}, and the dilution

concentration is 1.64×10^6 copies/ μ L, 1.64×10^5 copies/ μ L, 1.64×10^4 copies/ μ L, 1.64×10^3 copies/ μ L, 1.64×10^2 copies/ μ L, 1.64×10^1 copies/ μ L. See detailed instructions below:

- 1) Thaw the RCA DNA Control and DNA dilution buffer on ice. After completely thawed, vortex gently to mix, and centrifuge at low speed for 10 sec.
- 2) Take out six clean 1.5 mL tubes, marked with Std0, Std1, Std2, Std3, Std4, Std5.
- 3) Add 90 μ L DNA dilution buffer and 10 μ L RCA DNA Control to the 1.5 mL microfuge tube labeled Std0, namely dilute to RCA DNA Control concentration is 1.64×10^6 copies/ μ L. Mix and then centrifuge for 10 sec. Subpackage the diluted DNA standard and it can be stored in the short term (no more than 3 months) at $-25 \sim -15^\circ\text{C}$. Please avoid repeated freeze-thaw.
- 4) Add 90 μ L DNA dilution buffer into other tubes^{***}, then follow the below procedure for the serial dilutions^{****}.

Tube	Dilution Ratio	Standard concentration
Std1	10 μ L Std0 + 90 μ L DNA Dilution Buffer	1.64×10^5 copies/ μ L
Std2	10 μ L Std1 + 90 μ L DNA Dilution Buffer	1.64×10^4 copies/ μ L
Std3	10 μ L Std2 + 90 μ L DNA Dilution Buffer	1.64×10^3 copies/ μ L
Std4	10 μ L Std3 + 90 μ L DNA Dilution Buffer	1.64×10^2 copies/ μ L
Std5	10 μ L Std4 + 90 μ L DNA Dilution Buffer	1.64×10^1 copies/ μ L

Table1 Standard gradient dilution

^{*}Three replicate wells are required for each concentration. If required, the linear range may be expanded or reduced as appropriate.

^{**}To reduce the number of repeat freeze-thaw and avoid contamination, it is recommended to store the DNA control in aliquots at $-25 \sim -15^\circ\text{C}$ for the first time.

^{***}Once thawed, DNA dilution buffer could be stored at $2 \sim 8^\circ\text{C}$ for 7 days, if not used for a long time, please store at $-25 \sim -15^\circ\text{C}$.

^{****}Make sure the template is completely mixed, gently shake the mixture for 15 sec for each gradient dilution.

2. Test Sample (TS) preparation

Set the test sample (TS) in the experiment, the specific operation steps are as follows:

- 1) Add 100 μ L test sample into a clean 1.5 mL tube, then marked as TS.
- 2) Perform the DNA extraction of TS sample to prepare the purified TS sample.

3. Extraction Recovery Control (ERC) preparation

Set the concentration of RCA DNA standards in ERC as needed (the ERC sample was prepared with 1.64×10^4 copies RCA DNA as an example)^{*}, as follows:

- 1) Add 100 μ L test sample into a clean 1.5 mL tube, then add 10 μ L Std3 and mix well, marked as ERC.
- 2) Perform the DNA extraction of ERC sample together with the test samples to prepare the purified ERC sample.

^{*}It is generally recommended that the sample spiked amount be set at 0.8~1.5 times the actual content of the target gene RCA in the sample. If the content of the target gene in the sample is lower than the limit of quantification (LOQ), the spiked amount should be set to be within the LOQ, in order to ensure the reliability of the test results.

4. Negative Control Solution (NCS) preparation

Set the negative control in the experiment, the specific operation steps are as follows:

- 1) Add 100 μ L sample matrix (or DNA dilution buffer) into a clean 1.5 mL tube, then marked as NCS.
- 2) Perform the DNA extraction of NCS sample together with the test samples to prepare the purified NCS sample.

5. No Template Control (NTC) preparation

Set the no template control in the experiment, the specific operation steps are as follows:

- 1) NTC requires no sample pretreatment, and can be configured at the stage of qPCR detection of residual DNA.
- 2) The NTC sample in each tube or well is 20 μ L Mix (i.e. 15 μ L RCA qPCR Mix + 4 μ L RCA Primer&Probe Mix + 1 μ L IC) + 10 μ L DNA Dilution Buffer. It is recommended to configure three replicate wells.

6. PCR reaction system

Component	Volume(μ L)
RCA qPCR Mix [*]	15
RCA Primer&Probe Mix	4
IC	1
DNA Template	10
Total volume ^{**}	30

Table2 Reaction system

^{*}Calculate the total PCR reaction volume by the number of reactions: qPCR Mix =(the number of reactions+2) $\times$ (15+4+1) μ L (including the losses of two reaction wells). More than three replicates for each sample are recommended in the experiment.

^{**}After capping the tube or sealing the plate, centrifuge the reaction tube or plate at low speed for 10 sec. After sufficient shaking and mixing for 5 sec, repeat centrifuge to collect the liquid from the lid or wall to the bottom. Avoid bubbles during operation.

See below table for the recommended Plate setup:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC		TS 1	TS 1	TS 1		Std 1	Std 1	Std 1			
B	NTC		TS 2	TS 2	TS 2		Std 2	Std 2	Std 2			
C	NTC		TS 3	TS 3	TS 3		Std 3	Std 3	Std 3			
D							Std 4	Std 4	Std 4			
E	NCS		ERC 1	ERC 1	ERC 1		Std 5	Std 5	Std 5			
F	NCS		ERC 2	ERC 2	ERC 2							
G	NCS		ERC 3	ERC 3	ERC 3							
H												

Table3 Computer-on reference board

The plate layout includes: 5 Std (the standard curve of 5 standard concentrations), 1 NTC (no template control), 1 NCS (negative control solution), 3 TS (test samples), 3 ERC (extraction recovery control). Three replicate wells for each sample.

7. Setup guidelines for a PCR Instrument (2-step method) (eg. Thermo ABI 7500 qPCR instrument, Version 2.0 software)

The following instructions apply only to Thermo ABI 7500 qPCR instrument (Software version 2.0). If you use a different instrument, refer to the applicable instrument guide for setup guidelines.

- 1) Generate a new experiment, choose the template of absolute quantification or user-defined.

2) Create 2 detection probe, one named "RCA-DNA", select reporter fluorophore as "FAM" and quench fluorophore as "None", the other named "IC" and select the reporter fluorophore as "CY5" and quenching fluorophore as "None". The reference fluorescence is ROX" (the reference fluorescence can be based on the instrument model, etc., select whether you need to add it).

3) In the "Assign target (s) to the selected wells" pane, set the "Task" column to "Standard", and in the "Quantity" column, assign the FAM channel "164000", "16400", "1640", "164", "16.4" respectively; then in the corresponding "sample name" column named "164000 copies/μL", "16400 copies/μL", "1640 copies/μL", "164 copies/μL", "16.4 copies/μL". Set the task of NTC as NTC. Set the NCS, TS, and ERC as Unknown, and named them according to the above Plate layout correspondingly. Then click next.

4) Set the amplification program: set the reaction volume as 30 μL.

Cycle Step	Temperature(°C)	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	40
Annealing/Extension (Fluorescence collection)	60°C	30 sec	

Table4 Amplification procedure

8. Analysis of qPCR results

1) The system will automatically give the Threshold In the Amplification Plot panel of Analysis. The Threshold given by the system is too close to the baseline sometimes, resulting in a large difference in Ct between replicate wells. You can manually adjust the Threshold to an appropriate position and click Analyze. Then you can initially check whether the amplification curve is normal in Multicomponent Plot.

2) In the Result Analysis tab, review the Standard Curve plot. Verify the values for the R^2 , Efficiency, Slope and Y-intercept. For a normal standard curve, $R^2 > 0.99$, $90\% \leq \text{Eff\%} \leq 110\%$, $-3.6 \leq \text{Slope} \leq -3.1$.

3) In the "View well table" pane in Analysis, the concentrations of each samples are showed in Quantity, the unit is copies/μL, the units can be convert in the assay report.

4) The parameter settings of the result analysis need to be based on the specific model and the software version used, and can generally be automatically interpreted by the instrument.

5) Calculate the spike recovery rate based on the test results of the sample TS to be measured and the sample spike recovery ERC, the recovery rate of spikes is required to be between 50%~150%.Spiked recovery rate meter formula:

Recovery (%) = {Sample spiked assay (eg.copies/μL) - Sample assay (eg.copies/μL)} x Elution volume (μL) / Theoretical value of DNA addition amount (eg.copies) x 100%.

6) The Ct value of the negative control NCS should be greater than the mean of the lowest concentration Ct of the standard.

7) Template free control NTC should be Undetermined or Ct value ≥ 35 .

8) The Ct-IC value of the sample to be tested should be consistent with or ± 1 to the Ct-IC value of the NTC, and if the Ct-IC value of the sample to be tested is significantly larger compared to the Ct-IC value of the NTC, it indicates that the sample may be significantly inhibited. If spiked samples are tested at the same time, the sample spiked recovery results are prioritized and the IC results are used as a reference.

Notes

1. This product is for research use only.
2. Please operate with lab coats and disposable gloves, for your safety.
3. Please read this manual carefully before using this reagent, and the experiment should be standardized, including sample handling, reaction system preparation and sample addition.
4. Ensure that each component is fully vortexized and centrifuged at low speed before use.