

KRISHGEN® Residual SV40 LTA/E1A DNA Quantitation Real Time PCR Kit



Instruction Manual



KBPL9092



Store the kit and its components at -20°C

For Research Use Only

RUO

100 reactions

Introduction:

Residual SV40 LTA/E1A DNA Quantitation Real Time PCR Kit is used to quantitate residual SV40LTA & E1A host cell DNA, such as HEK293T cell. This kit uses duplex real-time PCR technique to perform rapid, specific, and reliable quantitation assay at the level of 10 copies/ul.

Intended Use:

The KRISHGEN® Residual SV40 LTA/E1A DNA Quantitation Real Time PCR Kit can be used to quantitate residual SV40LTA & E1A host cell DNA in different stages of biopharmaceutical products, from in-process samples to final products.

Kit Components:

1. Residual SV40 LTA/E1A linear DNA Control (lyophilized) – 1 vial
2. Residual SV40 LTA/E1A non-linear DNA Control – 50 ul x 1 vial
3. qPCR Reaction Buffer – 850 ul x 2 vials
4. Residual SV40 LTA/E1A Primer & Probe Mix – 300 ul x 1 vial
5. IPC Mix – 150 ul x 1 vial
6. DNA Dilution Buffer – 1.5 ml x 3 vials
7. Instruction Manual

Additional Requirements to be provided by the End-user:

1. DNase-free, Low Retention Microfuge Tubes, 1.5ml
2. Low Retention Tips of 1000 ul, 100 ul, 10 ul.
3. 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
4. Microcentrifuge
5. Vortex mixer
6. Micropipettes of 1000 ul, 100 ul, 10 ul
7. Real-time PCR instrument
8. Microplate shaker

Applied instruments, including but not limited to the following:

1. ABI 7500 Real-Time PCR System
2. CFX96 Real-Time PCR System
3. StepOne Plus Real-Time PCR System
4. Real Time PCR System, compatible

Stability and Storage:

Store the DNA Control, qPCR Mix and DNA dilution buffer at -20°C, protect from light.

General Precautions:

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.

- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) is available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.
Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Reagent Preparation:

Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, pipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.
3. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

DNA Control serial dilutions for the standard curve:

- Residual SV40 LTA/E1A DNA Control: Spin Residual SV40 LTA/E1A DNA Control tube for 15 seconds in a centrifuge and then add 55 ul of ddH₂O accurately to the bottom of the tube to dissolve the lyophilized powder.
- Gently flick the DNA Control standard solution with finger several times, then spin for 3-5 seconds in a centrifuge. Repeat 3 times to fully dissolve the lyophilized powder in the solution. Leave the tube stand for 10 min before use.

Calculations:

Plasmid copy numbers (copies/ul) = $6.02 \times 10^{14} \times$ Plasmid concentration (ng/ul) / (Number of plasmid bases x 660)

Residual SV40 LTA linear DNA Control: 4.67×10^9 copies/ul

E1A linear DNA Control: 4.97×10^9 copies/ul

Residual SV40 LTA non-linear DNA Control: 2.80×10^9 copies/ul

E1A non-linear DNA Control: 2.98×10^9 copies/ul

1. Thaw Residual SV40 LTA/E1A DNA Control and DNA Dilution Buffer completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge. Repeat this 3 times.
2. If Residual SV40LTA/E1A non-linear DNA Control is chosen, label seven nonstick 1.5 ml microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4 and ST5. If Residual SV40LTA/ E1A linear DNA Control is chosen, label eight nonstick 1.5 ml microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the Residual SV40 LTA/E1A DNA Control with DNA Dilution Buffer in the ST tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge and repeat 3 times.
4. Add 90 ul DNA Dilution Buffer to all tubes ST1, ST2, ST3, ST4, ST5 and ST6.
5. Perform the serial dilutions according to below table

Conc. (copies/ul)				Standard Vials	Dilution Particulars
Non-linear		Linear			
SV40 LTA	E1A	SV40 LTA	E1A		
2.80x10 ⁸	2.98x10 ⁸	4.67x10 ⁸	4.97x10 ⁸	ST	10 ul DNA Control + 90 ul DNA Dilution Buffer
2.80x10 ⁷	2.98x10 ⁷	4.67x10 ⁷	4.97x10 ⁷	ST0	10 ul ST + 90 ul DNA Dilution Buffer
2.80x10 ⁶	2.98x10 ⁶	4.67x10 ⁶	4.97x10 ⁶	ST1	10 ul ST0 + 90 ul DNA Dilution Buffer
2.80x10 ⁵	2.98x10 ⁵	4.67x10 ⁵	4.97x10 ⁵	ST2	10 ul ST1 + 90 ul DNA Dilution Buffer
2.80x10 ⁴	2.98x10 ⁴	4.67x10 ⁴	4.97x10 ⁴	ST3	10 ul ST2 + 90 ul DNA Dilution Buffer
2.80x10 ³	2.98x10 ³	4.67x10 ³	4.97x10 ³	ST4	10 ul ST3 + 90 ul DNA Dilution Buffer
2.80x10 ²	2.98x10 ²	4.67x10 ²	4.97x10 ²	ST5	10 ul ST4 + 90 ul DNA Dilution Buffer
-	-	4.67x10 ¹	4.97x10 ¹	ST6	10 ul ST5 + 90 ul DNA Dilution Buffer

- The remaining unused DNA Dilution Buffer need to be stored at 2-8°C. If the solution is cloudy or contains precipitates, heat at 37°C until it is clear.
- At least five concentration of standard curve should be included. To select appropriate sample dilutions, we recommend performing method validation before sample testing.

Sample Preparation:

Negative Control Sample (NCS) Preparation:

Take 100 ul of DNA Dilution Buffer to a new 1.5 ml clean centrifuge tube and label as NCS. NCS and samples should be prepared in same way for DNA extraction.

Assay Protocol:

A) qPCR Mix preparation:

1. Determine the number of reaction wells based on the standard curve, with the number of test samples and control samples. Generally, triplicates are tested for each sample.

Number of reaction wells = (5 standard points on the standard curve + 1 NTC + 1 NCS + test samples) x 3

2. Prepare qPCR MIX according to the number of reaction wells.

Reagent	Volume / reaction	Volume for 30 reaction (includes 10% coverage)
qPCR Reaction Buffer	15.9 ul	524.7 ul
SV40 LTA/E1A Primer & Probe Mix	2.8 ul	92.4 ul
IPC Mix	1.3 ul	42.9 ul
Total Volume	20 ul	660 ul

3. After thoroughly and place on ice, follow 20 ul each tube is divided into PCR 8-strip tubes or 96-well qPCR plate.

B) qPCR Reaction mix preparation:

1. Prepare qPCR Reaction mix according to Table below, and a 96-well plate layout template is shown in chart.

Tubes	Standard Curve	NTC	NCS	Test sample
qPCR Mix	20 ul	20 ul	20 ul	20 ul
Samples	10 ul ST1 – ST5	10 ul DDB	10 ul purified NCS	10 ul purified test sample
Total Volume	30 ul	30 ul	30 ul	30 ul

Chart: Reference 96-well plate layout.

S1	S1	S1										A
S2	S2	S2										B
S3	S3	S3							ST5	ST5	ST5	C
S4	S4	S4							ST4	ST4	ST4	D
S5	S5	S5							ST3	ST3	ST3	E
									ST2	ST2	ST2	F
NTC	NTC	NTC							ST1	ST1	ST1	G
NCS	NCS	NCS										H
1	2	3	4	5	6	7	8	9	10	11	12	

This example represents the assay for a standard curve with 5 concentration gradients (ST1-ST5), 1 NTC, 1 NCS, 5 test samples (S1 to S5), and 3 replicates for each sample.

In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity. Please refer to the example shown in chart.

Seal the 96-well plate with sealing film. Mix well in microplate shaker, then spin down the reagents for 10 seconds in microcentrifuge and place it on the qPCR instrument.

C) qPCR Program Settings:

NOTE: The following instructions apply only to the ABI7500 instrument with SDS v1.4. If you use a different instrument or software, refer to the applicable instrument or software documentation.

1. Create a new document, and then in the Assay drop-down list, select Standard Curve (Absolute Quantitation).
2. In the Run Mode drop-down list, select **Standard 7500**, and then click **next**.
3. Click New Detector, then enter SV40LTA-DNA in the Name field, select FAM in the Reporter Dye drop-down list and select (none) in the Quencher; Click New Detector, then enter E1A-DNA in the Name field, select CY5 in the Reporter Dye drop-down list and select (none) in the Quencher; Click New Detector, then enter IPC in the Name field. Select VIC in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK.
4. Select **ROX** as the passive reference dye, and then click **next**.
5. Select the applicable set of wells for the samples, and then select the corresponding detector for each well.
6. Select Finish, and then set thermal-cycling conditions:
 - a. Set the thermal cycling reaction volume to 30 ul.
 - b. Set the qPCR program as mentioned in the below table:

qPCR running temperature and time:

Step	Temp.	Time (mm:sec)	Cycles
Activation	95°C	10:00	1
Denaturation	95°C	00:15	40
Annealing/extension	60°C*	01:00	

*Instrument will read the fluorescence signal during this step.

7. Save the document, then click **Start** to start the real time qPCR run.

Calculation of Results:


1. Select **Set up** tab, then set tasks for each sample type by clicking on the Task Column drop-down list:

- a. NTC: target DNA detector task = **NTC**
- b. NCS, test samples: target DNA detector task = **Unknown**

2. Set up the standard curve as shown in table:

Settings for Standard curve

Tube Label	Task	Non-linear DNA (copies/ul)		Linear DNA (copies/ul)	
		SV40 LTA	E1A	SV40 LTA	E1A
ST1	Standard	2.80x10 ⁶	2.98x10 ⁶	4.67x10 ⁶	4.97x10 ⁶
ST2	Standard	2.80x10 ⁵	2.98x10 ⁵	4.67x10 ⁵	4.97x10 ⁵
ST3	Standard	2.80x10 ⁴	2.98x10 ⁴	4.67x10 ⁴	4.97x10 ⁴
ST4	Standard	2.80x10 ³	2.98x10 ³	4.67x10 ³	4.97x10 ³
ST5	Standard	2.80x10 ²	2.98x10 ²	4.67x10 ²	4.97x10 ²
ST6	Standard	-	-	4.67x10 ¹	4.97x10 ¹

- 3. Select the **Results** tab, and then select Amplification Plot.
- 4. In the Data drop-down list, select **Delta Rn vs Cycle**.
- 5. In the Analysis Settings window, enter the following settings:
 - a. Select **Manual Ct**.
 - b. In the Threshold field, enter 0.02.
 - c. Select **Automatic Baseline**.
- 6. Click the button  in the toolbar, and then wait the plate analyzing.
- 7. Select the **Result** tab > **Standard curve** tab, then verify the Slope, Intercept and R² values.
- 8. Select the Report tab, and then achieve the mean quantity and standard deviation for each sample.
- 9. Select **File > > Export > > Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.
- 10. In the Report panel of Results, the 'Mean Quantity' column can read the detection values of NTC, NCS, test sample.
- 11. The Ct value of IPC need to be analysed. In principle, the mean Ct-IPC value of the sample should be within ±1.0 of the NCS Ct-IPC value. If the mean Ct-IPC value of the sample is significantly higher than the NCS, this indicates that the sample may be inhibitory to the assay. If you have included ERC sample in parallel, then consider sample recovery rate prior to IPC results, and IPC results can only be used as reference.
- 12. The SV40 LTA detection value of NTC should be no more than 14.32 copies/ul, the E1A detection value of NTC should be no more than 17.79 copies/ul or set criteria by your own validation methods. The Ct value of NCS should be larger than the Ct value of the minimum standard curve concentration, and if the proven limit of quantification concentration is lower than the minimum standard curve concentration, the detected value of NCS should be less than the limit of quantification concentration.

Note: The parameter settings of the result analysis should be configured on the specific model and the software version, and generally can also be automatically interpreted by the instrument.

Symbols



Use by



Lot/Batch



Catalog number



Temperature limitation



Caution, consult accompanying documents



Manufacturer

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