EIV32025E

ExCell Bio

ResiQuant® Ready-to-use HEK293 DNA Size Analysis Kit (Taqman)

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number CRH00-1111

CRH00-1112

PRODUCT DESCRIPTION

The kit is used for the rapid detection of Human Embryonic Kidney 293 (HEK293) host cell DNA and it's fragment distribution in the intermediate, semi-finished and final products of various biologicals and drugs. Uracil N-glycosylase (UNG) is included in the kit to effectively degrade contamination possibly derived from the previous PCR product, thereby greatly reducing the false positive rate. ROX is suitable for ABI fluorescence quantitative PCR instrument or other similar equipment and plays the role of optical path correction.

In older to mitigate potential oncogenic and infectious risks associated with host cell DNA, the Center for Drug Evaluation (CDE) of Chinese National Medical Products Administration (NMPA) explicitly mandates in the *Guideline for Non-Clinical Research and Evaluation of In Vivo Gene Therapy Products (Trial)* that residual DNA fragment sizes must be controlled, with a recommended threshold of under 200 bp. This product targets primate-specific high-abundance conserved genomic sequences and incorporates host nucleic acid residue control requirements. It features four distinct amplicon-length detection systems designed to quantify residual host nucleic acids with the target length at 77 bp, 198 bp, 355 bp, 520 bp. It enables quantitative detection of both total DNA residues and the proportional distribution of residual fragments, specifically evaluating the percentage of nucleic acid residues exceeding 200 bp.

It is recommended to use the Universal DNA Residual Sample Pretreatment Kit for isolation and purification of the residual DNA from variant samples as in most cases the sample matrix can not be directly used in the detection assay. And for new type of sample matrix, proper suitability study is suggested to be done in advance to make sure the reliability of the test results.

PERFORMANCE, APPLICATION AND RESTRICTION

The kit is suitable for diverse samples from intermediates to final drug products with quantitative detection range from 300 pg/μL to 30 fg/μL.

| SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Components	CRH00-1111（50T）	CRH00-1112（100T）
293-Std1	150 µL	250 µL
293-Std2	150 µL	250 µL
293-Std3	150 µL	250 µL
293-Std4	150 µL	250 µL
293-Std5	150 µL	250 µL
293 Dilution Buffer	4 mL	4 mL × 2
2× 293 RTU qPCR Mix	750 µL × 4	1.5 mL × 4
6× 293 Detection Mix-77 bp	250 µL	500 µL
6× 293 Detection Mix-198 bp	250 µL	500 µL
6× 293 Detection Mix-355 bp	250 µL	500 µL
6× 293 Detection Mix-520 bp	250 µL	500 µL

Storage condition: -40℃ to -18℃.

Validity: 12 months under specified storage conditions.

Transportation: Dry ice.

Applicable instrument: ABI 7500, Bio Rad CFX-96, Agilent Mx3000P *et al*.

| EXPERIMENTAL PREPARATION

Instruments and reagents needed

1. Real-time qPCR instrument (FAM and HEX/VIC channels must be included. If "reference" option is available, please select "ROX").
2. Special pipettes and compatible low-retention pipette tips with filter elements.
3. Low-retention microtubes and PCR strips (qPCR-compatible).
4. Lab coats, disposable gloves, face masks, and other PPE.

Division of experimental area

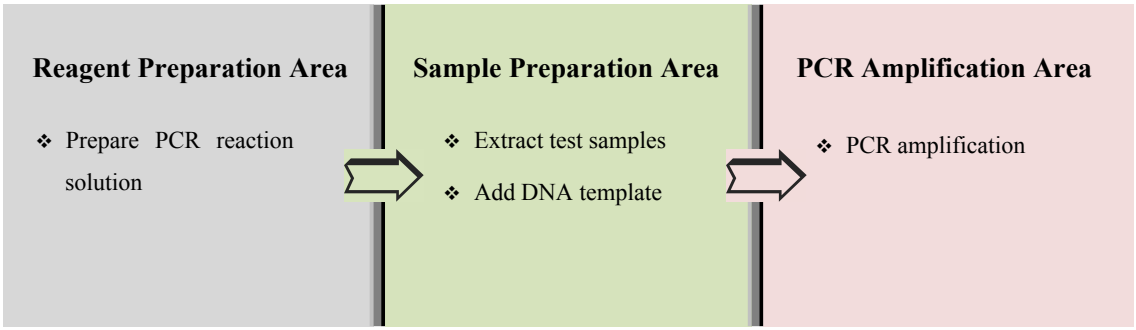
The following division of experimental area is recommended to avoid cross-contamination:

1. Reagent Preparation Area: A physically isolated space (e.g., laminar flow hood) dedicated to the reagents preparation except for samples.
2. Sample Preparation Area: A segregated workspace for nucleic acid extraction, dilution, and other sample-handling steps.
3. PCR Amplification Area: A region for PCR amplification that is independent to the first two regions.

EXPERIMENTAL PROCEDURE

Abbr.	Name	Description
NTC	No Template Control	Negative control
NEG	Negative Extraction Control	Pretreated, negative samples
TS	Test Sample	Sample to be tested
ERC	Extraction Recovery Control	Spiked samples

Operation process



Preparation of PCR reaction solution (Reagent Preparation Area)

For the first use, thaw all components and briefly centrifuge at 1000 × g for 10 sec to ensure reagents are collected at the tube bottom.

1. Determine the number of test samples and controls.
2. Reaction number = (five standard dilutions + one NTC + one NEG + TS + ERC) × 3.
3. Move 6× 293 RTU Detection Mix, 2× 293 RTU qPCR Mix to room temperature and briefly centrifuge.
4. Prepare a PCR reaction mix using the reagents and volumes shown in the table below. Add 20 µL PCR reaction mix to each well (Maintain at 2℃ to 8℃ prior to reaction initiation).

77 bp amplified fragment reaction system

Reagents	Volume for single reaction
2× 293 RTU qPCR Mix	15 μL
6× 293 Detection Mix-77	5 μL
Total	**20 μL**

198 bp amplified fragment reaction system

Reagents	Volume for single reaction
2× 293 RTU qPCR Mix	15 μL
6× 293 Detection Mix-198	5 μL
Total	**20 μL**

355 bp amplified fragment reaction system

Reagents	Volume for single reaction
2× 293 RTU qPCR Mix	15 μL
6× 293 Detection Mix-355	5 μL
Total	**20 μL**

520 bp amplified fragment reaction system

Reagents	Volume for single reaction
2× 293 RTU qPCR Mix	15 μL
6× 293 Detection Mix-520	5 μL
Total	**20 μL**

Note: *Use 10% excess volume to compensate for pipetting losses.*

Sample preparation (Sample Preparation Area)

DNA extraction:

It is recommended to use Universal DNA Residual Sample Pretreatment Kit to extract host-cell DNA from the samples.

Prepare the standard curve:

1. Move the tubes of 293-Std1 ~ 293-Std5 from the freezer and thaw at room temperature. Vortex gently and then briefly centrifuge.

Prepare the PCR plate (Sample Preparation Area)

Plate layout:

	77 bp			198 bp			355 bp			520 bp		
	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
B	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS
C	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS	TS
D	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5
E	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4
F	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3
G	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2
H	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1

1. Add 10 μL each of DNA template to the corresponding wells.
2. Carefully cover the PCR stripe and briefly centrifuge.

PCR amplification (PCR Amplification Area)

The following steps take the ABI 7500 fluorescence quantitative PCR instrument as an example:

1. Log in, and click “New Experiment” in the upper-left corner of the screen.
2. Enter the name of the experiment, Select “7500 (96 wells), “Quantitation-Standard Curve”, “TaqMan® Reagents” and “Standard”.
3. Click “Plate Setup”, and choose “FAM” as reporter and “None” as quencher. Add or change sample names if necessary.
4. Click “Assign Targets and Samples”, and set samples, NTCs, and standards in corresponding positions of the plate.

   Select “ROX” in the “Select the dye to use as the passive reference” column.

   Set up the standard serial dilutions with the following steps: (1) Click “Define and Setup Standards” (2) Enter “300” in blank after “Starting Quantity” (3) Choose “1:10” in “Serial Factor” (4) Select and arrange wells for the standards (5) Click “apply”.
5. Click “Run Method”, and set “Reaction Volume Per Well” as 30 μL, then set up the reaction procedure according to the following table:

Step		Temperature（℃）	Time（s）	Cycles
1	UNG Incubation	37	300	1
2	Initial Denaturation	95	180	1
3	Denaturation	95	15	40
	Annealing & Extension (Fluorescence Collection)	55	60	
The channel for HEK293: FAM				

6. After all settings are complete, click the green “Start Run” button.
7. After the run completes, select “Analysis” in the left.
8. Set the threshold to “Auto”, verify that the amplification curves exhibit normal sigmoidal shapes.
9. The slope, intercept and R^2 of the standard curve are shown on the Standard Curve interface.

Quality control

1. $R^2 \geq 0.98$, the slope of the standard curve should be between -3.60 to -3.10, and the amplification efficiency should be between 90% to 110%.
2. The analysis parameters should be set according to the software used, and this process can be automated by the instrument. NTC, NEG Ct ≥ 32 or No Ct.

Result judgment

The C_{Sample} represents the concentration of the test sample:

FAM	Description	Report
$Ct_{Sample} < Ct_{Std1}$	$C_{Sample} >$ 300 pg/μL, samples with concentrations above the ULOQ must be diluted to fall within the quantifiable range and reanalyzed.	/
$Ct_{Std1} \leq Ct_{Sample} \leq Ct_{Std5}$	For samples within the quantifiable range, calculate concentrations via the standard curve.	Calculate the concentration
$Ct_{Sample} > Ct_{Std5}$ or No Ct	Sample concentration below LLOQ.	$C_{Sample} <$ 30 fg/μL

The measured concentrations from the four detection systems were normalized to the 77 bp system (set as 100%), enabling calculation of the percentage of DNA residues exceeding 198 bp, 355 bp, and 520 bp.

Cautions

1. Wear disposable gloves, masks, and a clean lab coat.
2. Use calibrated pipettes (certified within the last 12 months).
3. Use low-retention filter pipette tips.
4. Use dedicated pipettes, pipette tips, and related equipment for each experimental area.

5. Vortex and briefly centrifuge (1000 × g, 10 sec) PCR solutions to collect reagents at the tube bottom.
6. Open and close all reagent/reaction tubes carefully to prevent cross-contamination.
7. Load samples in the following order: NTC, NEG, TS, and ERC.
8. Use separate pipettes for NTC, samples, and DNA template transfer to prevent contamination.
9. Avoid carrying PCR products into reagent preparation or sample preparation areas.
10. All workbenches and instruments must be cleaned with 75% alcohol after use.

DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.
2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.