

# ExCell Bio

## ResiQuant<sup>®</sup> Ready-to-use Quantitative HEK293 DNA Kit (Taqman)

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

### User Manual

Catalog Number CRH00-1081S

CRH00-1081

CRH00-1082



## | PRODUCT DESCRIPTION

The kit is used for the rapid detection of Human Embryonic Kidney 293 (HEK293) host cell DNA 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 that has already been through the PCR process, 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.

This product is suitable for residual host DNA detection using HEK293 cell lines like 293-T, 293-F, 293-H, 293-S. Five well established gradient DNA standards (range from 300 pg/μL to 30 fg/μL) are supplied with the kit as a set of ready-to-use references, simplifying the daily operation and lessening between-run variations.

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 cannot 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 different sample types from intermediates to finished products of biopharmaceuticals. with quantitative detection range from 300 pg/μL to 30 fg/μL.

## | SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Components	CRH00-1101 (50T)	CRH00-1102 (100T)	CRH00-1101S (50T)
293-Std1	150 μL	250 μL	150 μL
293-Std2	150 μL	250 μL	150 μL
293-Std3	150 μL	250 μL	150 μL
293-Std4	150 μL	250 μL	150 μL
293-Std5	150 μL	250 μL	150 μL
293 Dilution Buffer	4 mL	4 mL × 2	4 mL
2 × 293 RTU qPCR Mix	750 μL	750 μL × 2	750 μL
6 × 293 RTU Detection Mix	250 μL	500 μL	250 μL

**Storage condition:** -40°C to -18°C.

**Validity:** 12 months under specified storage conditions.

**Transportation:** Dry ice transportation.

**Applicable instrument:** ABI 7500, Agilent MX 3000P, Bio Rad CFX-96.

## | EXPERIMENTAL PREPARATION

### Instruments and reagents needed

- Real-time qPCR instrument (FAM and HEX/VIC channels must be included. If "reference" option is available, please select "ROX").
- Special pipettes and corresponding low adsorption pipette tips with filter element.
- Low adsorption 1.5 mL tube and strip PCR tubes (Adaptive to qPCR instrument).
- Clean lab clothes, disposable gloves, masks, etc.

### Division of experimental area

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

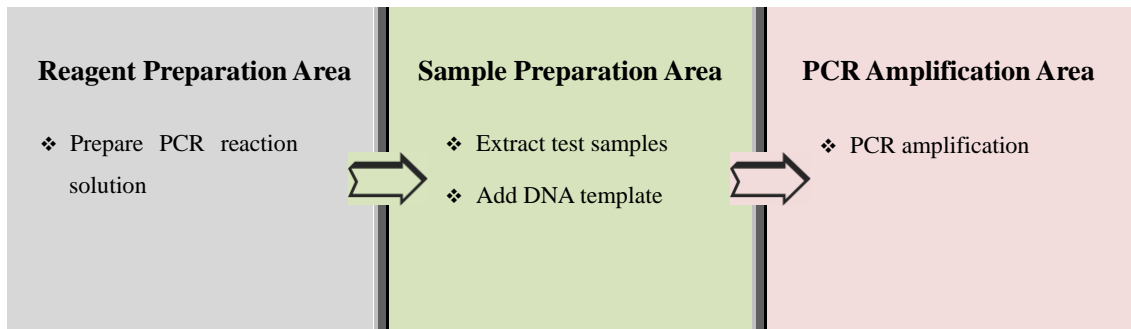
- Reagent Preparation Area: A separate preparation area for all other reagents except the samples, which can be a physical quarantine area such as a clean workbench.
- Sample Preparation Area: A separate preparation area for sample preparation, including extracting and diluting.
- PCR Amplification Area: Relatively independent from the first two areas for PCR amplification.

## | EXPERIMENTAL PROCEDURE

### Description

Abbr.	Name	Note
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, please thaw each component and centrifuge briefly to ensure that the reagent is collected at the bottom of the tube.

- Determine the number of test samples and controls:
- Reaction number = (five standard dilutions + one NTC + one NEG + TS + ERC) × 3
- Move 6 × 293 RTU Detection Mix, 2 × 293 RTU qPCR Mix to room temperature and spin shortly;
- Prepare a PCR reaction mix using the reagents and volumes shown in the table below. Add 20 µL PCR reaction mix to each well. (Keep between 2°C to 8°C before initiation of the reaction).

Reagents	Volume for single reaction
2 × 293 RTU qPCR mix	15 µL
6 × 293 RTU Detection Mix	5 µL
<b>Total</b>	<b>20 µL</b>

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

**Sample extraction (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 spin briefly.

## Prepare the PCR plate (Sample Preparation Area)

Plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC		TS1	TS1	TS1		TS1 ERC	TS1 ERC	TS1 ERC	
B					TS2	TS2	TS2		TS2 ERC	TS2 ERC	TS2 ERC	
C					TS3	TS3	TS3		TS3 ERC	TS3 ERC	TS3 ERC	
D	ST5	ST5	ST5									
E	ST4	ST4	ST4									
F	ST3	ST3	ST3						NEG	NEG	NEG	
G	ST2	ST2	ST2									
H	ST1	ST1	ST1									

1. Add 10  $\mu$ L each of DNA template to the appropriate wells.
2. Carefully cover the PCR stripe and centrifuge briefly.

### 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 top 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. Another reporter target is “VIC”, with “None” as the quencher. Add or change sample names if necessary.
4. Click “Assign Targets and Samples”, and set samples, NTCs, and standards in corresponding position of the plate.

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

Set up the standard serial dilutions with 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  $\mu$ L, then set up the reaction procedure according to the following table:

Step		Temperature ( $^{\circ}$ C)	Time (s)	Cycles
1	Digestion	37	300	1
2	Hold	95	180	1
3	Denature	95	15	40
	Anneal/extend	55	60	
The channel for HEK293: FAM				

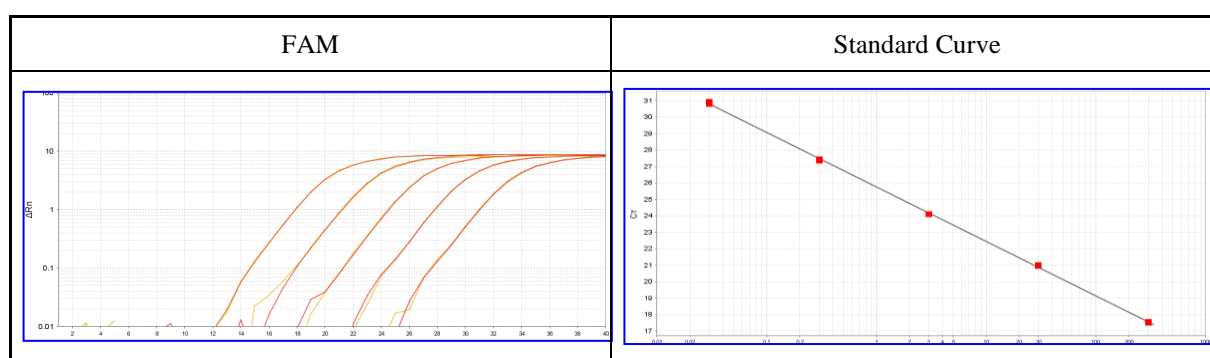
6. After all settings are complete, select the green button "Start Run".
7. When the procedure is finished, click "Analysis" on the left.
8. Set Threshold as Auto, and check whether the shape of the amplification curve is typical.
9. The slope, intercept and  $R^2$  of the standard curve are shown on the Standard Curve interface.

### Quality control

- The slope of the standard curve should be between -3.60 ~ -3.10, and the amplification efficiency should be between 90% ~ 110%.
- The analysis parameters should be set according to the software used, it can be automatically by the instrument. NTC, NEG Ct  $\geq$  32 or No Ct.

### Description of inspection results

Reference example



### Result judgment

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

FAM	Description	Report
$Ct < Ct_{Std1}$	$C_{Sample} > 300 \text{ pg}/\mu\text{L}$ , which the concentration of the test sample is over the Upper Limit of Quantification. It should be diluted to an appropriate concentration and then test again.	/
$Ct_{Std1} \leq Ct \leq Ct_{Std5}$	The concentration of the sample is within the quantitative range, and calculate the concentration according to the Standard Curve.	Calculate the concentration
$Ct > Ct_{Std5}$ or No Ct	The concentration of the test sample is less than the Lower Limit of Quantitation.	$C_{Sample} < 30 \text{ fg}/\mu\text{L}$

### Cautions

- Wear disposable gloves, masks, clean lab coat.
- Use calibrated pipettes.
- Use low adsorption pipette tips with a filter.
- Use dedicated pipettes, pipette tips and related equipment in different experimental Areas.
- Please vortex and centrifuge the PCR solution briefly to ensure that the reagent is collected at the bottom of the tube.
- Be careful in opening or closing all reagent or reaction tubes to avoid cross contamination.
- Load samples sequentially as following: NTC, NEG, TC and ERC.
- Use dedicated pipettes to separately transfer NTC, samples and DNA template in order to avoid

contamination.

- Clean bench and instrument surfaces with 75% alcohol after the test.
- Discard the used pipette tips in 0.1% sodium hypochlorite solution and clean the whole site after the experiment.

## | 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.