EIV32025E

# ExCell Bio

# ResiQuant® Quantitative Vero DNA Kit (Taqman)

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

## User Manual

| | |
|---|---|
| Catalog Number | CRH00-1031S |
| | CRH00-1031 |
| | CRH00-1032 |

## PRODUCT DESCRIPTION

The kit is used for the rapid detection of African green monkey kidney (Vero) 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 possibly derived from the previous PCR product, thereby greatly reducing the false positive rate. The reagent components include an internal control (IC) and reference dye (ROX). The signal performance of IC allows monitoring of the reaction process to exclude sample interference. ROX is suitable for ABI fluorescence quantitative PCR instrument or other similar equipment and plays the role of optical path correction.

This product is designed for the detection of residual host DNA from the African green monkey (Vero) host cells. To obtain the standard curve, the Vero DNA Control is used to prepare the standards by serial dilution (range from 300 pg/μL to 3 fg/μL).

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 3 fg/μL.

## SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

| Components | CRH00-1031（50T） | CRH00-1032（100T） | CRH00-1031S（50T） |
|---|---|---|---|
| Vero DNA Control | 20 μL | 40 μL | 20 μL |
| DNA Dilution Buffer | 4 mL | 4 mL × 2 | 4 mL |
| 2× Vero qPCR Mix | 750 μL | 750 μL × 2 | 750 μL |
| 6× Vero Detection Mix | 250 μL | 500 μL | 250 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

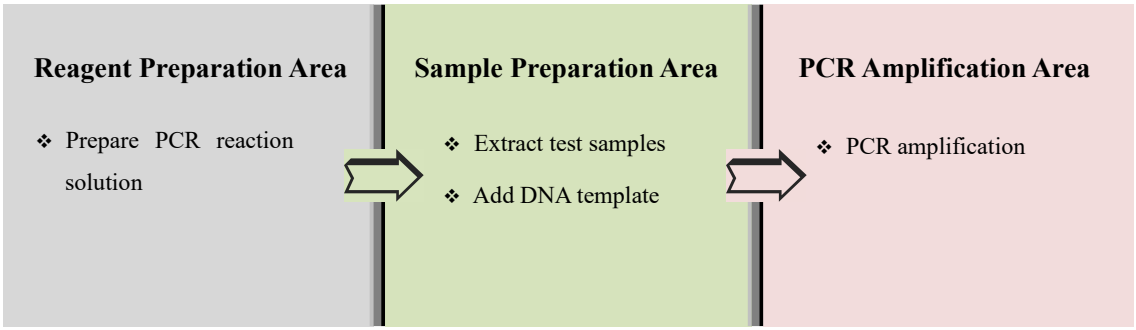
The following division of experimental area is recommended to avoid cross-contamination:

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 = (six standard dilutions + one NTC + one NEG + TS + ERC) × 3.
3. Move 6× Vero Detection Mix, 2× Vero 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).

| Reagents | Volume for single reaction |
|---|---|
| 2× Vero qPCR Mix | 15 µL |
| 6× Vero Detection Mix | 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. Label low-retention 1.5 mL microtubes: ST0, ST1, ST2, ST3, ST4, ST5, ST6 (ST0 will not be used for the standard curve).
2. Add 90 µL DNA Dilution Buffer to the tubes ST1 to ST6 (Use new tips for each tube).
3. Thaw Vero DNA Control (30 ng/µL) completely at room temperature, thoroughly mix reagents, and briefly centrifuge.

4. Dilute Vero DNA Control to 3 ng/μL: calculate the required volume of DNA Dilution Buffer and Vero DNA Control, transfer a certain volume of DNA Dilution Buffer into the ST0 tube, add an appropriate amount of Vero DNA Control, fully vortex and mix well, and quickly centrifuge.
5. Perform the serial dilutions as described in the following table (Use new tips for each tube):

| Tube | Dilution | Concentration |
|---|---|---|
| ST1 | 10 μL ST0 + 90 μL DNA Dilution Buffer | 300 pg/μL |
| ST2 | 10 μL ST1 + 90 μL DNA Dilution Buffer | 30 pg/μL |
| ST3 | 10 μL ST2 + 90 μL DNA Dilution Buffer | 3 pg/μL |
| ST4 | 10 μL ST3 + 90 μL DNA Dilution Buffer | 300 fg/μL |
| ST5 | 10 μL ST4 + 90 μL DNA Dilution Buffer | 30 fg/μL |
| ST6 | 10 μL ST5 + 90 μL DNA Dilution Buffer | 3 fg/μL |

***Note:*** *The DNA Dilution Buffer can be stored at 2℃ to 8℃ and used within 2 months, -40℃ to -18℃ out of 2 months.*

## 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 | ST6 | ST6 | ST6 | | 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 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. 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 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 | 300 | 1 |
| 3 | Denaturation | 95 | 15 | 40 |
| | Annealing & Extension (Fluorescence Collection ) | 60 | 60 | |
| The channel for Vero: FAM； The channel for IC: HEX/VIC | | | | |

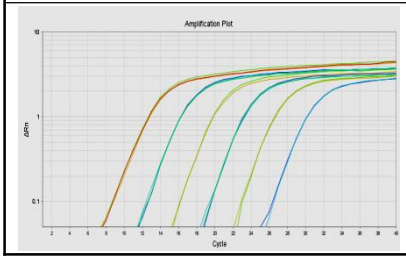
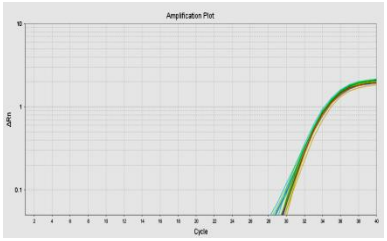
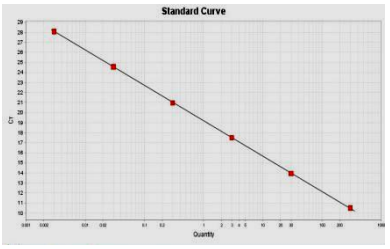
6. After all settings are complete, click the green “Start Run” button.
7. After the run completes, select “Analysis” in the left.
8. Set the threshold to “Auto”, verify that the amplification curves exhibit normal sigmoidal shapes.
9. The slope, intercept and $R^2$ of the standard curve are shown on 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 Ct of IC: CV ≤ 5%.
3. 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.

## Description of inspection results

Reference example

| FAM | HEX/VIC | Standard Curve |
|---|---|---|
|  |  |  |

## Result judgment

The△Ct (HEX/VIC) in the table is the difference between the Ct of sample and the mean Ct of the calibrators,

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

| FAM | HEX/VIC | 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 | / |
| $Ct_{Std1} \leq Ct_{Sample} \leq Ct_{Std6}$ | △Ct ＜ -1 | The reaction fluid is not uniformly mixed or there is matrix effect | / |
| | -1≤△Ct ≤1 | For samples within the quantifiable range, calculate concentrations via the standard curve | Calculate the concentration |
| | △Ct ＞ 1 | The reaction fluid is not uniformly mixed or here is matrix effect | / |
| $Ct_{Sample}$ ＞ $Ct_{Std6}$ or No Ct | / | Sample concentration below LLOQ | $C_{Sample}$ ＜ 3 fg/μL |

## 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.