

Add the following:**▲〈509〉 RESIDUAL DNA TESTING****INTRODUCTION**

The following method is suitable for measurement of residual host cell DNA in recombinant therapeutic products produced in either *Escherichia coli* (*E. coli*) or Chinese hamster ovary (CHO) cell lines. Also provided is an extraction procedure that is suitable to be used prior to a quantitative polymerase chain reaction (qPCR)-based method for the measurement of residual host cell DNA. For discussion of the principles and best practices for this type of testing, see *Nucleic Acid-Based Techniques—Approaches for Detecting Trace Nucleic Acids (Residual DNA Testing)* 〈1130〉, which may be a helpful resource.

PROCEDURE**• SAMPLE PREPARATION**

There are several procedures for isolating DNA that may be appropriate for biopharmaceutical sample testing. One such procedure is described in detail below and validated for starting DNA concentrations ranging from 0.01 to 50 pg/μL.

Resuspension solution: Dissolve tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and ethylenediaminetetraacetic acid (EDTA) to obtain a solution of 10 mM and 1.0 mM, respectively. Add hydrochloric acid or sodium hydroxide to adjust to a pH of 8.0.

DNA standard stock solution: Dilute either the USP CHO Genomic DNA RS or the USP *E. coli* Genomic DNA RS, as appropriate, to a concentration of 1 μg/mL in *Resuspension solution*.

Sample solutions: Samples for testing may require dilution or reconstitution to: 1) overcome matrix interference affecting the DNA recovery, 2) yield an appropriate starting volume, or 3) bring the analyte concentration within the quantitative range of the qPCR method. *Sample solutions* may be diluted in water or in *Resuspension solution* if necessary. For drug substance samples, *Sample solutions* should contain sufficient starting material to allow determination of the residual DNA content, if present at the specification limit.

Positive control solution: Prepare by spiking *DNA standard stock solution* to *Sample solutions* at a concentration appropriate for the assay (specification, or otherwise justified).

Negative control solution: Water or *Resuspension solution* is used in place of *Sample solutions* in the extraction procedures and will be extracted with any samples (if extraction is necessary). The *Negative control solution* is tested using the qPCR-based method to determine the DNA content contributed by the background and to demonstrate that there is no potential cross-contamination during the assay.

70% Ethanol: Prepare by adding water to ethanol to give a final concentration of 70% (v/v).

Proteinase K solution: Dissolve Tris-HCl, EDTA, and sodium dodecyl sulfate (SDS) to obtain solutions of 100 mM (pH 8.0), 5 mM, and 100 mg/mL, respectively. Add proteinase K¹ to this solution to a final concentration of 10 mg/mL.

Sodium iodide solution: 6 M sodium iodide, 15 mM EDTA, 0.25% sodium sulfite, 0.5% sodium *N*-lauroyl sarcosinate, 25 mM Tris-HCl pH 8.0, and 35 μg/mL linear acrylamide. For 100 mL (scale as appropriate), while mixing with a stir bar on a magnetic plate, add in the following order: 50 mL of nuclease-free water, 0.25 g of sodium sulfite, 3.0 mL of 0.5 M EDTA solution, and 2.5 mL of 1 M Tris-HCl pH 8.0; then slowly add 89.93 g of sodium iodide. Add nuclease-free water to a final volume of 100 mL. Filter the solution using a 0.2-μm nylon membrane. [NOTE—This stock solution can be stored in the dark at 4° for up to 1 year.] On the day of use, add linear acrylamide and sodium *N*-lauroyl sarcosinate to final concentrations of 35 μg/mL and 0.5% (w/v), respectively. [NOTE—Addition of sodium *N*-lauroyl sarcosinate to sodium iodide solution may result in turbidity. If so, warming to 50–55° for 2–3 min is helpful before using for extraction.]

Extraction: Add 50 μL of *Proteinase K solution* to 450 μL of triplicate samples each of *Sample solutions* and *Positive control solution*, and at least one sample of *Negative control solution*, in a 2.0-mL centrifuge tube. Mix and incubate at 60 ± 2° for 1–24 h. Add 500 μL of *Sodium iodide solution* to each tube. Mix and incubate at 40° for 15 min. Add 900 μL of 100% ethanol, mix well, and incubate at room temperature for 15 min. Centrifuge at 10,000 × g for 15 min to pellet the DNA. [NOTE—Centrifugation at 2°–8° may be helpful to improve DNA recovery.] Remove and discard the supernatant. Wash the pellet by adding 1 mL of 70% ethanol containing 3 μg/mL linear acrylamide, centrifuge again, and discard the supernatant. Air dry the pellet for 5–10 min until no visible liquid is observed. Resuspend the pellet and record the volume of water or *Resuspension solution* used, which may be needed when reporting the final results in *Calculations*.

• qPCR ANALYSIS

2X Master mix: A suitable buffer containing magnesium chloride, deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, deoxyuridine triphosphate, deoxythymidine triphosphate, and highly purified *Thermus aquaticus* DNA polymerase.² Mix well immediately before use.

DNA stock primers and probes: According to the DNA of the species tested, prepare individual 10-μM solutions of the following primer pairs and probe, using DNase-free water (see *Table 1*).

Table 1

<i>E. coli</i>	Forward primer	5'-CCTTACGACCAGGGCTACACA-3'
	Reverse primer	5'-CTCGCGAGGTCGCTTCTC-3'
	Probe ^a	5'-CGTGCTACAATGGCGCATACA-3'

¹ Sigma catalog #P2308 or a suitable alternative.

² ThermoFisher catalog #4440038 or a suitable alternative.

Table 1 (continued)

CHO	Forward primer	5'-CCTGAGTTCAATCCCAGCA-3'
	Reverse primer	5'-ACATTCTGCTTCCATGTATATCTGCA-3'
	Probe ^a	5'-TGGCTCACAACCATCCGTTATGAGACCT-3'

^a The probe can be 5'-labeled with 6-carboxyfluorescein and 3'-labeled with 6-carboxytetramethylrhodamine or a suitable alternative.

DNA probe solution: Dilute *DNA stock probe* to 2.5 µM with DNase-free water.

Standard solutions: Dilute the *DNA standard stock solution* to obtain five or more suitable standards within the concentration range of 0.001–100 pg/µL.

Analysis

Samples: *Sample solutions*, *Positive control solution*, *Negative control solution*, and *Standard solutions*

[NOTE—If samples are extracted, then extracted *Sample solutions* and extracted *Control solutions* will be used.]

Transfer 25 µL of the *2X Master mix* to each well of a 96-well qPCR plate. Add 5 µL each of the *DNA stock forward primer*, the *DNA stock reverse primer*, and the *DNA probe solution* of the appropriate species to each well. Add 10 µL of either (extracted) *Sample solutions*, *Standard solutions*, (extracted) *Negative control solution*, or (extracted) *Positive control solution* to their respective wells. [NOTE—The qPCR reaction volume may be scaled as appropriate to accommodate different instruments.] Mix, seal the plate tightly, and centrifuge for 1 min at 1000 × g. Place the plate in a suitable qPCR thermal cycler. Incubate for 2 min at 50°, then for 10 min at 95°, followed by 40 cycles, with each cycle consisting of 95° for 15 s and 60° for 1 min. [NOTE—Some instruments and reagents require a preincubation step. Carefully follow specific instrument/reagent recommendations.] Monitor the signal of the labeled probe using a suitable fluorescent detector. Determine the threshold value using the instrument-specific recommendations. Record the cycle thresholds (*C_t*) for each sample.

Calculations

Plot the log quantity of DNA of the *Standard solutions* versus the *C_t*.

Calculate the slope and the intercept.

Using these values and the following equation, calculate the quantity of DNA in each well:

$$\text{Result} = 10^{\left(\frac{C_t - b}{m}\right)}$$

C_t = cycle threshold of the *Sample solutions*

b = intercept of the line for the *Standard solutions*

m = slope of the line for the *Standard solutions*

Calculate the quantity of DNA in each of the *Sample solutions*. Correct for any dilution or concentration of the sample.

System suitability

Samples: *Negative control solution* and *Standard solutions*

Suitability requirements

Negative control solution: The *C_t* corresponding to the *Negative control solution*, if any, is NLT the *C_t* of all of the lowest concentrations of the *Standard solutions*.

Sensitivity: The *C_t* corresponding to the lowest concentration of the *Standard solutions* is NMT 39.

Linearity: The regression coefficient associated with the *Standard solutions* is NLT 0.98. The slope is between –3.1 and –3.8.

Acceptance criteria

Any measurable sample must fall within the standard curve.

Accuracy: The mean spike recovery of three replicates of *Positive control solution* is between 50% and 150%. [NOTE—If needed due to the extraction procedure, correct for the sample dilution.]

Relative standard deviation: NMT 30% for three replicates of the *Sample solutions*; NMT 30% for three replicates of *Positive control solution*

The limit of residual DNA is defined in the product monograph.

ADDITIONAL REQUIREMENTS

• USP REFERENCE STANDARDS (11)

USP CHO Genomic DNA RS

USP *E. coli* Genomic DNA RS▲ (USP 1-Dec-2019)