Description

The Residual Sf9 DNA Detection qPCR Kit is designed for the sensitive detection of trace Sf9 DNA in a sample using quantitative PCR (polymerase chain reaction). By using a specific primer and probe set that targets a SINE (short interspersed nuclear elements) consensus sequence in Sf9, this kit can achieve a detection limit as low as 1 fg/µl while minimizing background noise. Each kit provides enough 2x qPCR Master Mix (no dye) for 100 reactions and includes an Sf9 primer and probe mix, positive control, dilution buffer, and ROX reference dye for generating reliable standard curves.

Background

The manufacture of biological drugs, including vaccines, antibodies, and recombinant proteins, is regulated by agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to ensure that biopharmaceutical products do not contain harmful impurities. Most biologics are prepared using host cell systems such as human cells, insect cells, or bacterial cells, and carry-over of host cell DNA must be kept to a minimum as it could compromise patient safety. Therefore, manufacturers must ensure that the amount of residual host cell DNA is kept within safety levels defined by regulatory agencies. While specific requirements may vary depending on the host cell type and route of administration, these agencies generally recommend a maximum limit of 10 ng of host cell DNA per drug dose.

To facilitate quality control, the Residual Sf9 DNA Detection qPCR Kit enables the detection of Sf9 DNA at concentrations as low as 1 fg/µl, with no cross-reactivity to *E. coli*, HEK293, or CHO DNA. This specificity is achieved by targeting a consensus sequence constructed from short interspaced nuclear elements (SINEs) specific to the *Spodoptera frugiperda* species. SINEs are non-autonomous transposable elements that occur in high copy numbers throughout the genome, making them ideal targets for DNA detection. Although SINEs are found in many eukaryotic organisms, primer and probe specificity has been validated by BLAST analysis to be exclusive to the *Spodoptera* genus.

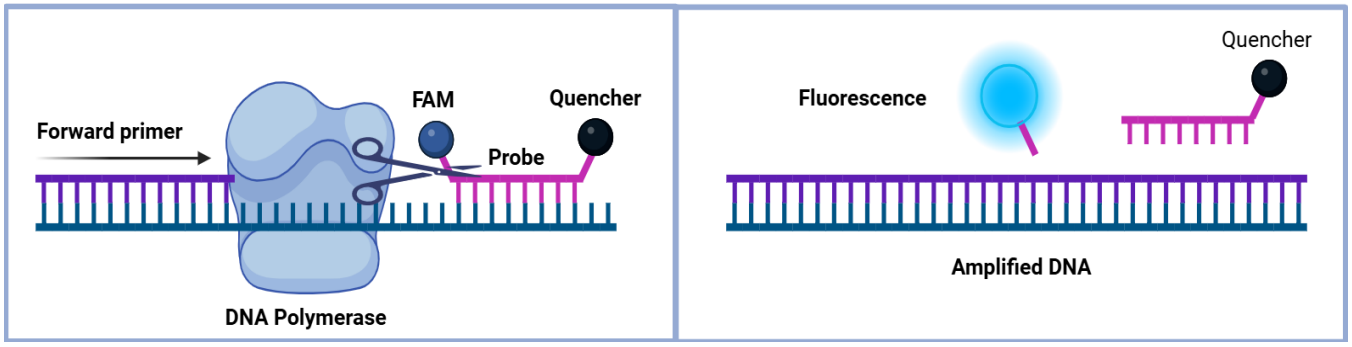


Figure 1. Principle of Probe-Based qPCR.
Detection utilizes a 6-FAM–labeled probe paired with Black Hole Quencher-1 (BHQ-1). (**A**) In the intact probe, fluorescence is quenched via Förster resonance energy transfer (FRET). (**B**) During the extension phase, the 5'-3' nuclease activity of Taq polymerase cleaves the probe, separating the 6-FAM fluorophore from the quencher to generate a fluorescent signal. The absorbance and emission maxima of 6-FAM are 495 nm and 520 nm, respectively.

Applications

- *Spodoptera frugiperda* DNA quantification in biological samples, including antibodies and recombinant proteins produced in Sf9 and Sf21 cell lines. Research Use Only.

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Supplied Materials

Catalog #	Name	Amount	Storage
93979-KC1.2+	2x qPCR Master Mix (no dye)	1.2 ml	-20°C
84373-KC220+	Sf9 Primer & Probe Mix	220 µl	-20°C
84374-KC20+	Sf9 DNA Control (10 ng/µl)	20 µl	-20°C
83737-KC600+	Dilution Buffer	600 µl	RT
93982-KC1+	DNA/RNAse-Free Water	1 ml	RT
83580-KC40+	ROX Dye (25 µM)	40 µl	-20°C

Storage Conditions

This kit will perform optimally for up to **6 months** from the date of receipt when the materials are stored as directed. We advise keeping the freeze-thaw cycles of the 2x qPCR Master Mix (no dye) to 5 cycles or less.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Materials Required but Not Supplied:

- CFX Connect Real-Time PCR Detection System (no probes required) or similar system (BioRad #1855201)
- 0.2 ml sterile qPCR tubes or 96 well qPCR plate
- 1.5 ml sterile microcentrifuge tubes
- Adjustable micropipette and sterile tips

Protocol

qPCR General Guideline

- Set up all reactions in duplicate and on ice.
- Limit light exposure to the Sf9 Primer/Probe Mix and the ROX dye when in use.
- The suggested quantity of ROX Reference Dye to include in the Master Mix differs depending on the type of qPCR instrument you are using.
    - For equipment that does not require ROX: No dye is needed
    - For low ROX instruments: use 1.5 µl of ROX Reference Dye per 600 µl of Master Mix
    - For high ROX instruments: use 15 µl of ROX Reference Dye per 600 µl of Master Mix

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Reference Dye	PCR Instrument
Low ROX (30nM)	Applied Biosystems®: 7500, 7500 Fast, ViiA™7, Quant-Studio™ instruments Stratagene (Agilent): MX4000P, MX3000P, MX3005P
High ROX (300nM)	Applied Biosystems®: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
No ROX required	BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX Opus, CFX-96 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCyler® Roche: LightCycler® 480, LightCycler® 2.0

- The assay should include the Sf9 DNA Control as the Positive Control and a No Template Control to determine the Limit of Detection (LOD).

qPCR Set-up

1. Prepare a serial dilution of Sf9 DNA control following the table below. This will be used to generate the standard curve.

Dilution Series	Volume of Control or previous dilution (µl)	Volume of Dilution Buffer (µl)	Concentration
Dilution 1	5 µl of Sf9 DNA Control	45 µl	1 ng/µl
Dilution 2	5 µl of Dilution 1	45 µl	100 pg/µl
Dilution 3	5 µl of Dilution 2	45 µl	10 pg/µl
Dilution 4	5 µl of Dilution 3	45 µl	1 pg/µl
Dilution 5	5 µl of Dilution 4	45 µl	100 fg/µl
Dilution 6	5 µl of Dilution 5	45 µl	10 fg/µl

2. Set up the qPCR reactions with the 6 dilutions prepared and a No Template Control (NTC) in duplicate in a qPCR 200 µl tube or a 96-well PCR plate using the following volumes:

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Component	Test Sample	1 ng	100 pg	10 pg	1 pg	100 fg	10 fg	NTC
Test Sample	X µl	-	-	-	-	-	-	-
Dilution 1	-	1 µl	-	-	-	-	-	-
Dilution 2	-	-	1 µl	-	-	-	-	-
Dilution 3	-	-	-	1 µl	-	-	-	-
Dilution 4	-	-	-	-	1 µl	-	-	-
Dilution 5	-	-	-	-	-	1 µl	-	-
Dilution 6	-	-	-	-	-	-	1 µl	-
Dilution Buffer	-	-	-	-	-	-	-	1 µl
Probe & Primer Mix	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
2X qPCR Master Mix (No dye)	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
DNA/RNAse-free water	8 - X µl	7 µl	7 µl	7 µl	7 µl	7 µl	7 µl	7 µl
Total	**20 µl**	**20 µl**	**20 µl**	**20 µl**	**20 µl**	**20 µl**	**20 µl**	**20 µl**

3. Run qPCR reactions using the following parameters:

Step	Temperature	Duration	Cycles
Denaturation	98°C	2 minutes	1x
Annealing	98°C	5 seconds	40x
Extension*	58.5°C	30 seconds	

*Capture FAM signal at the end of each extension step.
Note: These parameters have been optimized for use with CFX Connect Real-Time PCR Detection System (Bio-Rad). Further optimization may be required for other instruments.

4. Set the baseline threshold to a constant value above the background noise or use the auto-baseline feature to obtain Ct values.

5. If the Ct value of your No Template Control is less than the Ct value of Dilution 6 (10 fg), do not use this data to calculate the amount of residual DNA in your test sample and repeat the experiment using a fresh serial dilution of the Sf9 DNA control.

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Data Analysis

1. Plot the Ct value (Y-axis, linear scale) vs. Sf9 DNA in fg (X-axis, logarithmic scale) for the Sf9 DNA reference standard curve.

2. Apply logarithmic regression to determine the amount of residual Sf9 DNA in the unknown sample using the equation $y = mx + b$ from the logarithmic trendline equation you obtain from the standard curve.

   Note: The R^2 value should be > 0.95.

   Sf9 DNA = $10^{(Ct-b)/m}$, where **m** is the slope of the line and **b** is the y-intercept.

   For Example: if the trendline equation is y = -3.36 log(x) + 37.91; **Ct** of test sample = 30.00, the amount of Sf9 DNA (fg) is = $10^{(30.00-37.91)/-3.36}$ = 226.03 fg per reaction.

3. To calculate the amount of DNA per µl of test sample, one needs to account for the 20 µl reaction volume and the amount of test sample used for the reaction:

   $$\text{Amount of Sf9 DNA per µl of test sample} = \frac{\text{[Amount of DNA calculated for reaction (fg per reaction)]}}{\text{[Amount of test sample added (µl) x reaction volume(µl)]}}$$

   For Example:

   $$\text{fg/µl} = \frac{\text{226.03 fg per reaction}}{\text{2 µl x 20 µl per reaction}}$$

   fg/µl = 5.65 fg/µl of test sample

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Validation Data

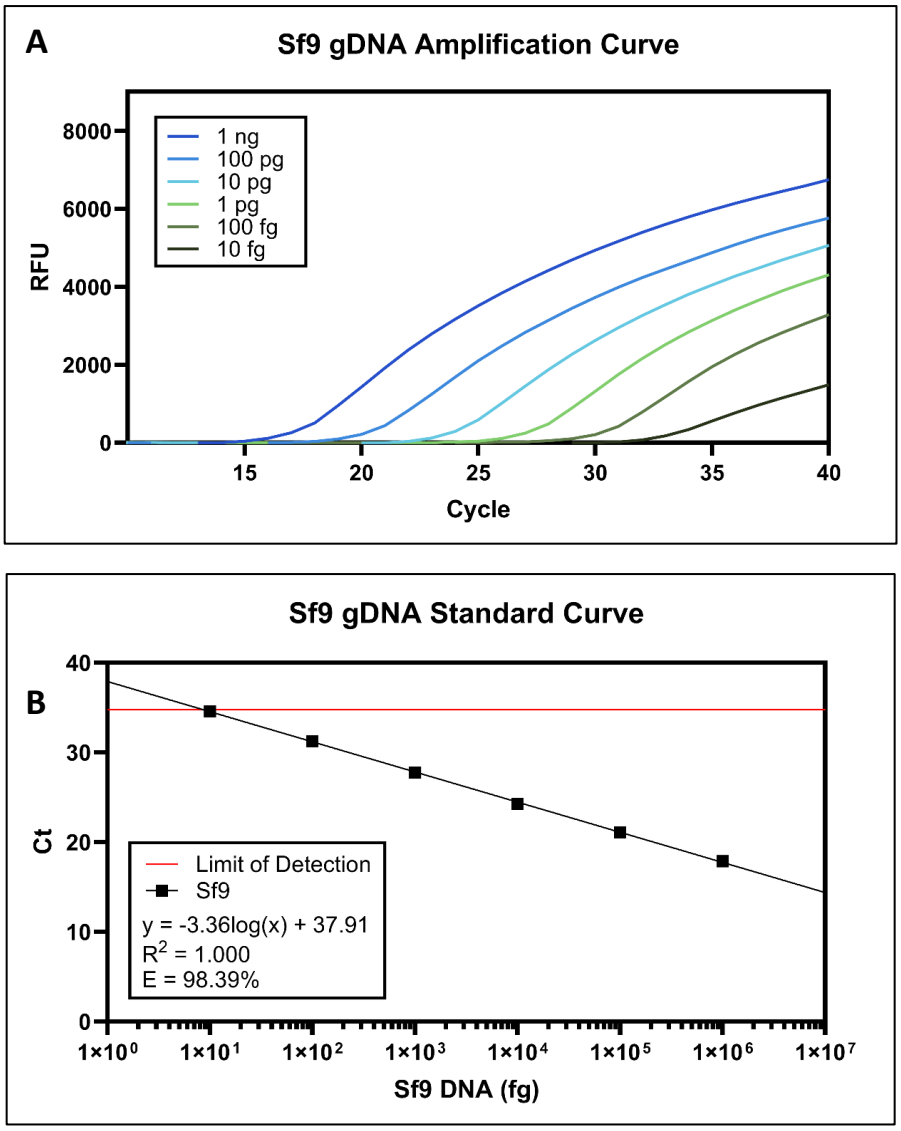
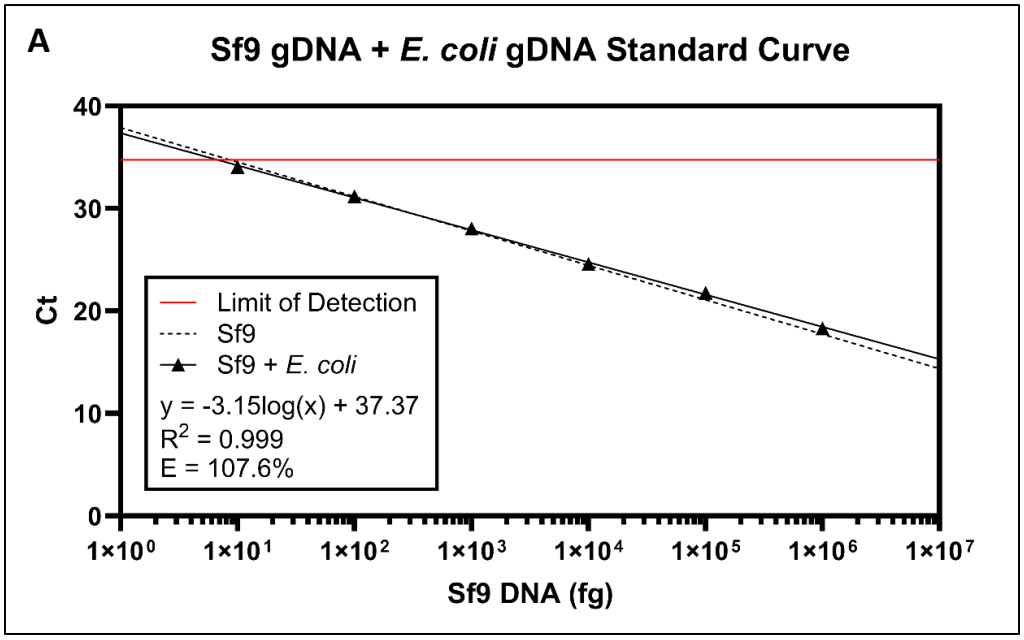
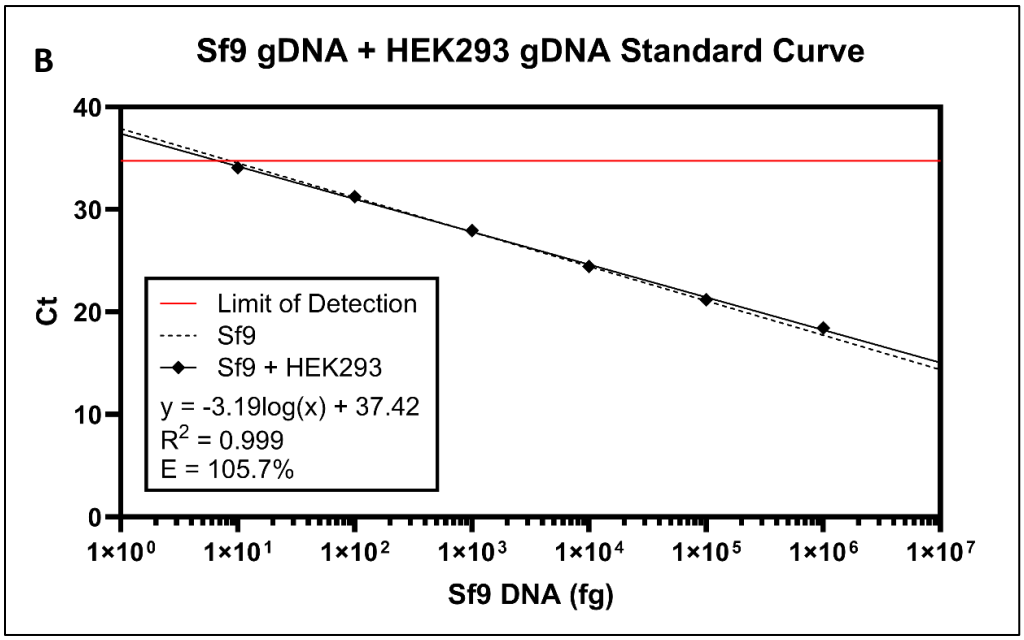


Figure 2. Sf9 genomic DNA amplification and standard curves.
(A) qPCR results showing the amplification curves of a serial dilution of the Sf9 DNA Control in the 1 ng – 10 fg range, in triplicate. The qPCR was run using primers targeting a SINE sequence derived from *Spodoptera frugiperda*, a FAM/BHQ1 reporter probe, and a dye-free 2x qPCR mix. **(B)** A standard curve was generated using an average of the Ct values from the serial dilution and the logarithmic trendline equation and limit of detection are shown.

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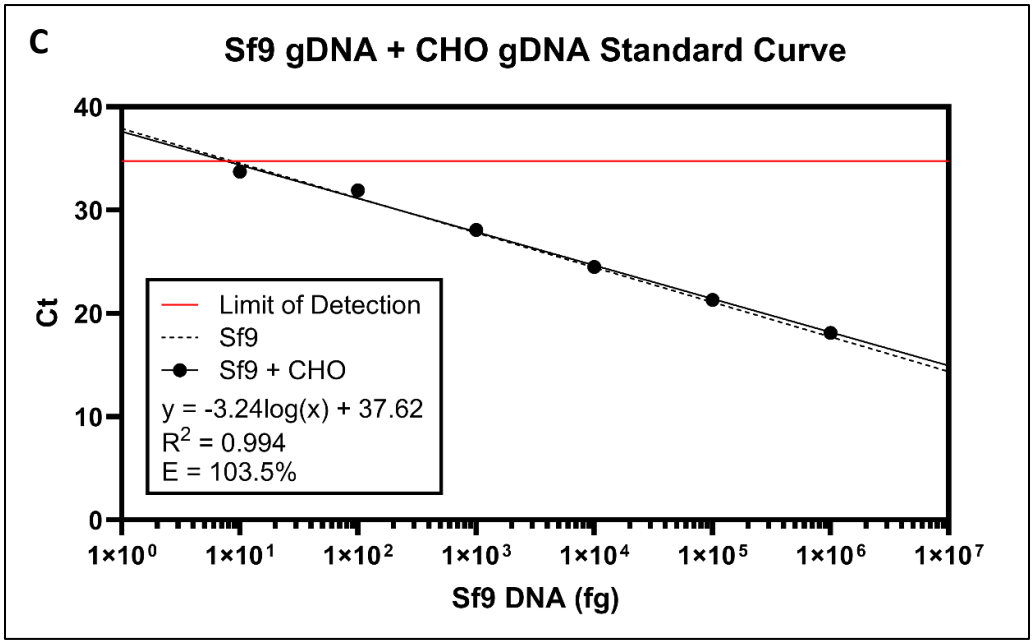


Figure 3. Sf9 genomic DNA standard curve in the presence of E. coli, HEK293, and CHO genomic DNA.
Sf9 DNA standard curves were generated from a serial dilution in the 1 ng – 10 fg range in the presence of 1 ng of genomic DNA from *E. coli* **(A)**, HEK293 **(B)**, or CHO **(C)**. Results show no cross-reactivity with other DNA.

Data shown is representative.

Troubleshooting Guide
For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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