CHO Host Cell DNA Kit

REF: KBBR02

Pack size: 100 Tests

Ver.2.1

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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KRISHGEN BioSystems 15375 Ashley Ct, Whittier, CA 90603
Tel: (213) 291 3096 Fax: (213) 402 2025 Email: sales_usa@krishgen.com

Customer Care and Technical Support

If you have any queries, do not hesitate to contact us. We thank you for your comments and advice.

- **For technical queries:** molbio@krishgen.com
- **For ordering:** sales@krishgen.com

This protocol handbook is included in:

Krishgen Biosystems CHO Host Cell DNA Kit

Background

For the production of biotechnological products, expression of therapeutic proteins in CHO cells is commonly used. Due to which there is a possibility of DNA contamination from the host cells in the products which are manufactured. However, the limit of DNA contamination from the cell lines has been set by regulatory agencies According to WHO the limit of DNA contamination should not exceed 10ng/dose in the products manufactured. One of the most common method which is widely used for detection of host cell DNA contamination by biopharmaceutical manufacturers is the PicoGreen™ Dye based assay. The research and development department at Krishgen Biosystems has designed a kit to detect and quantify host cell DNA in the products manufactured by recombinant expression in CHO cell lines based on PicoGreen™ Dye assay.

Principle

The Krishgen CHO Host Cell DNA Kit is based on the DNA dye binding assay which utilizes PicoGreen™ dye. The DNA samples and the standards are reacted with PicoGreen™ dye, which is a DNA interculator that binds strongly to the double stranded DNA. Upon binding to DNA, PicoGreen™ dye fluoresces with an excitation of 485 nm and emission of 525 nm. The intensity of the fluorescent signal is proportional to the quantity of DNA in the standard or the samples.

Material Provided

DNA Extraction Reagents	
*Buffer AL (Lysis solution)	35ml
*Buffer PP (Protein Precipitation Solution)	6mlx2
PicoGreen Assay	
CHO DNA Standards 10 µg/ml	100µl
TE buffer (20X)	12ml
PicoGreen™ dsDNA reagent	100µlx2
Assay Plate with Plate Sealing Foil	2 x 96 wells
Instruction Manual	1

**Buffer AL and PP may precipitate at cold temperature. If so, dissolve it in 37°C water bath.*

Materials Required By the End User

Reagent

- Isopropanol
- 70% ethanol
- Optional RNase solution (not provided)

Disposable material

- Sterile 1.5ml microcentrifuge tubes
- RNase free pipette tips

Equipments

- Micro centrifuge (4°C)
- Adjustable and properly calibrated pipettes to measure volumes ranging from 10µl to 1000µl.
- Vortex
- Heating Block; 37°C and 65°C
- Appropriate personal protector (e.g. lab coat, disposable gloves, goggles etc.).
- Flurometer

Quality Control

CHO Host Cell DNA Kit is manufactured in strict clean condition and its degree of cleanness for the components provided is monitored periodically. For quality control, the quality certification process is carried out thoroughly and only the qualified is delivered.

Storage Conditions

1. Buffer AL should be stored at 2-8 °C (15-25°C). A precipitate can be formed in Buffer AL under cool condition. In such case, heat the bottle at 56°C until the precipitate completely dissolves.
2. Buffer PP should be stored at 2-8°C and bring to room temperature when in use.
3. The PicoGreen*TM* dsDNA reagent is light sensitive and should be stored in the dark until use at -20 °C.
4. CHO DNA standards should be stored at -20 °C.
5. All the components in the kit are stable until the expiration date mentioned in the kit.

Precautions

1. The DNA extraction reagents, Buffer AL and PP contain irritant which is harmful when it comes in contact with skin or eyes or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.
2. The PicoGreen™ Solution is a DNA intercalating dye and should be handled with care. When working with PicoGreen™ Solution, use gloves, eye protector and mask to avoid contact with skin or clothing and inhalation of vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

CHO Host Cell DNA Detection and Quantification Protocol

NOTE:

1. Bring the PicoGreen™ solution to room temperature before proceeding with the experiment.

2. The PicoGreen™ Solution will bind to all double stranded DNA. It is important to keep the working area clean to avoid contamination by DNA in the environment. Thoroughly clean pipettes and the immediate working area before initiating the protocol.

3. Always make sure the centrifuge is balanced to ensure proper assay performance. An improperly balanced centrifuge can result in loose pellets, which can adversely affect recovery and assay precision

Reagent Preparation

TE buffer (1X)

To make TE (1X) buffer, add 1 ml of TE (20X) buffer to 19 ml of double distilled water.

Preparation of PicoGreen™ Solution

Dilute the PicoGreen™ solution in the ratio of 1:200. For example, if the experiment has 20 samples, add 30 µl of PicoGreen™ to 5970 °C µl of prewarmed (37ºC) TE Buffer. Mix by gently vortexing for 5 seconds.

Note: PicoGreen™ Solution must be diluted fresh for each experiment.

Standards

Upon first use, thaw 10 µg/ml tube of standard and quick-spin, aliquot into polypropylene vials, and store at -20°C. To run the assay, thaw and dilute the DNA by adding 20µl of standard solution in 980 ul of TE buffer (1X) to prepare the top standard (200 ng/ml).

Protocol for Extraction of DNA (Sample Preparation)

1. **Dilute all test samples to concentrations within the analytical range of the assay and to < 20mg/ml total protein using TE buffer. All samples should be diluted at least 1:2.**

2. **Transfer 300 µl of Buffer AL to a fresh 1.5ml microcentrifuge tube.**

3. **Add 300µl of sample to the tube containing buffer AL and pipette 5 to 6 times to resuspend thoroughly. Incubate the lysate at 37°C until clumps disappear.**
   Generally, cell lysis is completed in 5 min. However, complete resuspending of the cells is crucial for good DNA yield. If the clumps are still visible after 1 hour, add an additional 100µl of Buffer AL and repeat incubation.

4. ***Optional: If RNA-free DNA is required, add 1µl of RNase solution (20mg/ml) to the lysate and mix the sample by inverting the tube 5 times. Incubate the mixture for 15 min at 37°C.***

5. **Cool the sample to room temperature. Apply 100µl of Buffer PP to the mixture and vortex vigorously for 15sec. Centrifuge for 2 min at 14,000 x g.**

6. ***Optional: Incubate the sample on ice for 5min before centrifugation. This may increase the yield of DNA.***
   A dark brown protein pellet should be visible.

7. **Carefully transfer the supernatant to a fresh 1.5ml micro centrifuge tube containing 300 µl of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA forms a visible mass.**
   Do not vortex after addition of isopropanol.

8. **Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 300 µl of 70% ethanol (room temperature). Gently invert the tube several times to wash the DNA pellet and the side walls of the tube.**

9. **Centrifuge at 14,000 x g for 1 min. Carefully discard the ethanol by aspirating or pipetting. Invert the tube on clean absorbent paper and air dry the pellet for 10 to15 min.**
   The DNA pellet is very loose at this point and care must be taken to avoid missing the pellet. Ethanol should be completely removed, but over drying will make the rehydration of DNA pellet difficult.

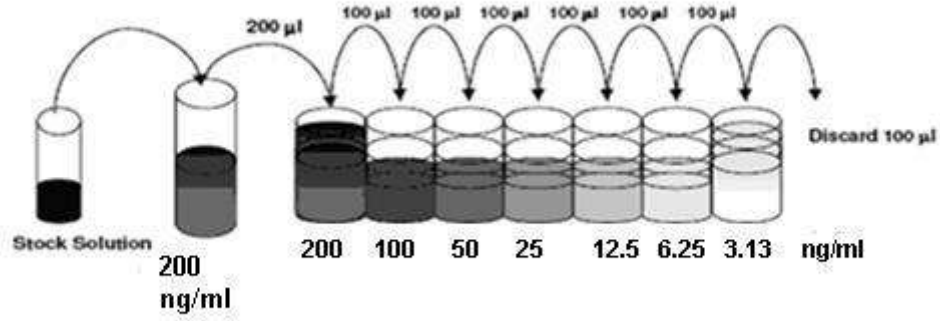
Protocol for Detection and Quantification of CHO Host Cell DNA

1. **Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.**

2. **Add 100 µl of Standards and Samples to the microcentrifuge tube. Further perform six two-fold serial dilutions of the 200 ng/ml top standard, in separate tubes. Thus, the CHO DNA standard concentrations are 200 ng/ml, 100 ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.13ng/ml and TE buffer (1X) serves as the zero standard (0ng/ml).**

   (Note: If you want to check sensitivity on your end you can dilute samples further. Please check our justification summary)

3. **Add PicoGreen™ in 1:1 proportion to standards. e.g. Add 100 µl of pre-diluted and pre-warmed PicoGreen™ reagent to the 100 µl of Standards. For samples directly add 250 µl of PicoGreen™ reagent in the DNA Pellet obtained and mix to dissolve the pellet. Remember to run your each standard and sample in duplicate.**



4. **Vortex each tube for 5 seconds and incubate for 5 minutes at Room Temperature under dark.**

5. **Remove 200µl from each tube and pipette into the Assay Plate (provided). Seal the plate with the foil provided and incubate for 5 minutes at room temperature.**

6. **Read the plate at Excitation wavelength: 485nm, Emission wavelength: 525nm, Cutoff: 515nm.**

Calculation of Results

1. The standards may be used to construct a standard curve with values reported in ng/ml host cell DNA.

2. This data reduction may be performed through computer methods using curve-fitting routines such as point-to-point, cubic spline and 4 parameter logistic fit. **Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies.**

Recommendation

1. Precision on triplicate samples should yield average % coefficients of variation of less than 15% for samples in the range of 500pg/ml to 150ng/ml.

2. Coefficients of variation for samples above or below this range (500pg/ml to 150ng/ml) may be greater than 15%.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Precision

DNA samples were prepared in a Human IgG matrix at various concentrations spanning the Standard Curve. Four preparations were made for each sample and duplicate wells were collected for each preparation.

Concentration	% Nominal	Intra-assay CV%	Inter-assay CV%
100ng/ml	90%	2.4%	9.8%
10ng/mL	94%	3.4%	7.5%
1ng/mL	98%	11.1%	11.0%

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