KRIBIOLISA® CHO Host Cell DNA (Picogreen) Kit

REF : KBBR02

Pack Size: 100 Tests

Ver 1.3

RUO

DNA Assay Kit for Accurate Quantitation of CHO Host Cell DNA from cell culture supernatant and other biological preparations

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

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REF KBBR02 Σ 100 tests

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Introduction:

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 10 ng/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 Private Limited 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.

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 intercalator 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.

Materials Provided:

DNA Extraction Reagents	
*Buffer AL (Lysis solution)	35 ml
Picogreen Assay	
CHO DNA Standard (65 ug/ml)	35 ul
Picogreen dsDNA reagent	30 ul
TE Buffer (10X)	10 ml
Assay Plate with Plate Sealing Foil	1 x 96 wells
Instruction Manual	1

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

Materials Required By the End User:

Reagent

1. Isoamyl Alcohol
2. Chloroform
3. Phenol
4. 70% ethanol.
5. Isopropanol
6. Optional RNase solution (not provided)

Disposable Material

1. Sterile 1.5 ml microcentrifuge tubes
2. RNase free pipette tips

Equipment

1. Micro centrifuge (4°C).
2. Adjustable and properly calibrated pipettes to measure volumes ranging from 10 ul to 1000 ul.
3. Vortex.
4. Heating Block; 37°C and 65°C.
5. Appropriate personal protector (e.g. lab coat, disposable gloves, goggles etc.)
6. Fluorimeter.

Handling/Storage

1. Buffer AL should be stored at room temperature (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 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.

Health Hazard Warnings

1. All the reagents provided may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing Use only.

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.

Procedural Notes

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 (all reagents should be diluted immediately prior to use):

1. **TE buffer (1X)**
   To make TE (1X) buffer, add 2 ml of TE (10X) buffer to 18 ml of double distilled water.

2. **Preparation of Picogreen Solution**
   Dilute the Picogreen solution in the ratio of 1:600. For example, if the experiment has 20 samples, add 8.3 ul of Picogreen to 4991.7 ul of pre-warmed (37°C) TE Buffer. Mix by gently vortex for 5 seconds.
   Note: Picogreen Solution must be diluted fresh for each experiment.

3. **Preparation of PP Buffer**
   PP buffer is prepared by adding highly pure Phenol:Chloroform:Isoamyl Alcohol in the ratio of 25:24:1 and the pH is adjusted to 8.05- 8.35 and should be stored at amber bottles.
   Note: At 15 -30°C the buffer will appear as a one clear solution (homogeneous phase).

Sample Preparation: Protocol for Extraction of DNA

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

2. Transfer 300 ul of Buffer AL to a fresh 1.5 ml micro centrifuge tube.

3. Add 300 ul of sample to the tube containing buffer AL and pipette 5 to 6 times to resuspend thoroughly.

4. Incubate the lysate at 37°C until clumps disappear.

Note: 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 100ul of Buffer AL and repeat incubation.

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

6. Cool the sample to room temperature. Add 100 ul of Buffer PP to the mixture and vortex vigorously for 15sec. Centrifuge for 2 min at 14,000 x g.
Note: 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 1.5 ml micro centrifuge tube containing 300 ul of isopropanol (room temperature) and gently mix the solution by inversion until the white thread-like strands of DNA forms a visible mass.
Note: Do not vortex after addition of isopropanol.

8. Centrifuge at 14,000 x g for 1 min. Decant the supernatant and add 300 ul 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.
Note: 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.

Assay Procedure:

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. **Standards Preparation:** Dilute 4.6 ul of original Standard (65 ug/ml) with 295.4 ul of standard diluent to generate a 1000 ng/ml Standard stock solution. Thus, the *CHO* DNA standard concentrations are 1000 ng/ml, 500 ng/ml, 250 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 15.63 ng/ml, 7.8 ng/ml and TE buffer (1X) serves as the zero standard (0ng/ml). Prepare the Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
1000 ng/ml	Standard No.8	4.6 ul Standard Provided (65 ug/ml) + 295.4 ul TE buffer (1X)
500 ng/ml	Standard No.7	150 ul Standard No.8 + 150 ul TE buffer (1X)
250 ng/ml	Standard No.6	150 ul Standard No.7 + 150 ul TE buffer (1X)
125 ng/ml	Standard No.5	150 ul Standard No.6 + 150 ul TE buffer (1X)
62.5 ng/ml	Standard No.4	150 ul Standard No.5 + 150 ul TE buffer (1X)
31.25 ng/ml	Standard No.3	150 ul Standard No.4 + 150 ul TE buffer (1X)
15.63 ng/ml	Standard No.2	150 ul Standard No.3 + 150 ul TE buffer (1X)
7.8 ng/ml	Standard No.1	150 ul Standard No.2 + 150 ul TE buffer (1X)
0 ng/ml	Standard No.0	Only TE buffer (1X)

3. Add 150 ul of Standards and Samples to the micro centrifuge tube.
Note: If you want to check sensitivity on your end you can dilute samples further. Please check our justification summary

4. Add Picogreen in 1:1 proportion to standards. E.g. Add 150 ul of pre-diluted and pre-warmed Picogreen reagent to the 150 ul of Standards. For samples directly add 150 ul of Picogreen reagent in the DNA Pellet obtained and mix to dissolve the pellet. Remember to run your each sample and standards in duplicate.

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

6. Remove 200 ul 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.

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

Calculation of Results:

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

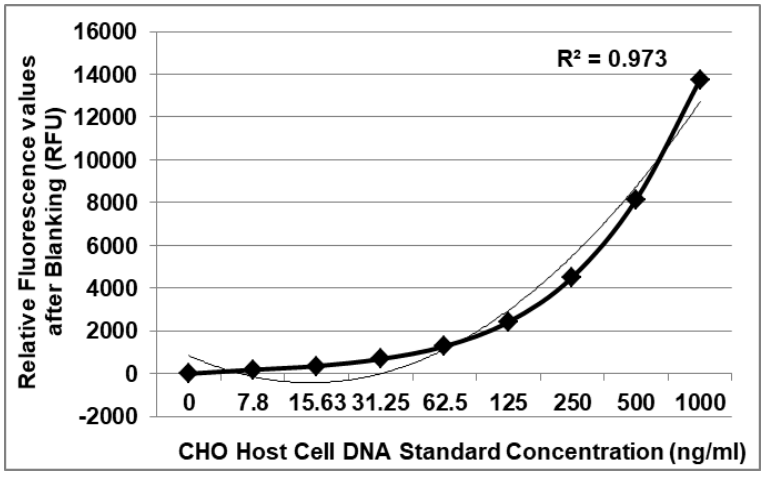
Note:

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%.

Typical Data (representative only)

CHO Host Cell DNA Standard Concentration (ng/ml)	Relative Fluorescence values (RFU)	Relative Fluorescence values after Blanking (RFU)
0	184	0
7.8	368	184
15.63	539	355
31.25	869	685
62.5	1461	1277
125	2598	2414
250	4692	4508
500	8321	8137
1000	14116	13748

Typical Graph (representative only)



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.
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

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.

Pool	Concentration	% Nominal	Intra-assay CV%	Inter-assay CV%
Low	7.8 ng/ml	98%	10.1%	10.0%
Medium	62.5 ng/ml	95%	3.2%	7.4%
High	1000 ng/ml	92%	2.3%	9.7%

Safety Precautions:

- This kit is **For Research Purposes only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (<0.1 % w/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **human body fluids** or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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