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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 34777-2017

**Detection of residual DNA from Chinese hamster ovary (CHO)
cells in biological products - Fluorescence quantitative PCR
method**

中国仓鼠卵巢（CHO）细胞表达产品残留DNA检测 荧光定量PCR法

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009. This standard was proposed by the National Biochemical Test Standardization Technical Committee (SAC/TC387) and centralized. This standard drafting unit. Cinda biological pharmaceutical (Suzhou) Co., Ltd., China Institute of Tested Technology Institute of Biology. The main drafters of this standard. Sun Zuoyu, Zhou Lihua, Li Xuefeng, Huang Xiaogang, Yuan Zhijun, Guo Yanhong. Chinese Hamster Ovary (CHO) Cells Express Products Residual DNA Detection Fluorescent Quantitative PCR

1 Scope

China's national method for measuring residual host cell DNA in biological products made in Chinese hamster ovary cells, by real-time quantitative PCR. It applies to the detection of host DNA in products expressed from CHO cell lines. CHO cells are the workhorse of the biopharmaceutical industry: most therapeutic monoclonal antibodies and a great many recombinant proteins are made in them, because they fold and glycosylate human proteins in a way bacteria cannot. The product is then purified out of a broth that also contains the cells that made it, and some of their DNA comes along. Regulators limit how much may remain - the concern is that host cell DNA carries oncogenic and infectious potential, and the limits are set in nanograms per dose, well below what any ordinary method would see. Quantitative PCR reaches those levels because it amplifies a specific repeated sequence in the CHO genome and counts the cycles needed to detect it, which is a logarithmic measurement and therefore usable across the whole range.

This standard specifies the detection of residual host DNA by fluorescence quantitative PCR method. This standard applies to the CHO (Chinese Hamster Ovary cells) host cell line expressed in the biological products of the detection of the host DNA.

2 Normative references

The following documents for the application of this document is essential. For dated references, only the dated version applies to this article Pieces. For undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682 analytical laboratory water specifications and test methods

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 Ct value cyclethreshold The number of cycles experienced when the fluorescent signal in each reaction tube reached a set threshold.

4 Abbreviations

The following abbreviations apply to this document. CHO. Chinese Hamster Ovary

5 Principle

In the eukaryotic cell genome with high copy number of 16SrRNA gene, designed to specifically amplify the gene PCR Forward primer, ForwardPrimer (5'-CCAGGCATTGGTGGCAC-3 ') and reverse primer, ReversePrimer (5'-AGACAGGGTTTCTCTGT-3 '). The SYBR fluorescence quantitative PCR (FQ-PCR) method was used to acquire the Ct value of the PCR amplification curve drawn by the fluorescence and was used in the amplification system The original purpose of the template is inversely proportional to the logarithm of the initial template DNA logarithm of the vertical axis, Ct value of the abscissa, making a standard curve. According to the standard curve equation to calculate the sample of DNA residues, so as to quantitatively detect the content of residual DNA samples purpose.

6 Equipment

6.1 Fluorescence quantitative PCR instrument containing SYBR fluorescent dye detection channel.

6.2 UV - visible spectrophotometer. Wavelength range .200nm ~ 760nm.

6.3 Microcentrifuge. Speed range..2000r/min ~ 23000r/min.