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PHARMACEUTICAL INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

YY/T 1876-2023

Tissue engineered medical products - Quantification of remnant DNA in animal-derived biomaterials - Fluorescent staining method

组织工程医疗产品 动物源性生物材料DNA残留量测定法：荧光染色法

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1 Scope

YY/T 1876 specifies how to measure the DNA left behind in an animal-derived biomaterial, using a fluorescent staining method. Decellularised tissue, collagen, pericardium and bone matrix taken from animals are made safe by stripping out the donor cells, and residual DNA is the accepted indicator of how completely that was done: too much of it and the graft risks an immune reaction in the recipient. The document fixes how the sample is prepared, how the stain is applied and how the reading is converted into a residual DNA figure, so that results from different laboratories can be compared and a release specification can be enforced. For a manufacturer of xenogeneic implants or tissue-engineered scaffolds, this is the assay a Chinese reviewer expects the decellularisation validation and the batch release data to be based on.

This document specifies a method for the determination of remnant DNA in biological materials utilizing animal tissues and their derivatives. This document applies to final products or intermediate products of biological materials utilizing animal tissues and their derivatives, scaffold materials utilizing animal tissues and their derivatives used as matrices or scaffolds for tissue engineered medical products, and can also be used for human-derived decellularized matrix materials.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

YY/T 0771.1, Medical devices utilizing animal tissues and their derivatives - Part

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1 extracellular matrix; ECM Produced by cells and secreted into the extracellular space within tissues, it includes a homogeneous matrix (proteoglycans and glycoproteins) and filamentous collagen fibers. It has the function of connecting and supporting cells and is the basic framework

4 Test principle

This document designs a three-step method for the quantitative detection of remnant DNA in biological materials utilizing animal tissues and their derivatives, namely, proteinase K digestion and/or tissue homogenization of solid biological materials, DNA purification, and DNA detection by fluorescent staining. In addition to the test sample, a spiked recovery test sample must be set up simultaneously (i.e., a certain amount of DNA control substance is added to the test sample). In general, nucleic acid extraction usually uses one or more of the following methods. cell lysis, tissue homogenization, enzymatic hydrolysis and ultrasonication. In order to obtain ideal purified DNA, it is recommended to use a combination of enzymatic hydrolysis and/or tissue homogenization. This combined method is particularly suitable for situations where decellularized ECM materials are difficult to digest. In order to avoid the interference of digested decellularized ECM components on DNA detection, use a nucleic acid extraction kit to purify DNA. In this document, nucleic acid dyes (such as Picogreen fluorescent dye) are used for the quantitative detection of double- stranded DNA.

5 Samples, reagents, and instruments

5.1 Test sample Sample requirements are as follows:

a) Test sample. Decellularized matrix materials (final products or semi-finished products) and raw materials shall be sterilized and virus inactivated in accordance with YY/T 0771.1 and YY/T 0771.3 to ensure biosafety;

b) DNA standard curve samples. Use animal cell-derived DNA control samples to establish a standard curve;

c) DNA purification spike recovery sample. Add a certain amount of DNA control substance to another sample with the same amount as the sample to be tested, and use it to determine the spike recovery rate;

d) Negative control sample. Composed of phosphate-buffered saline (PBS) and bovine serum albumin (BSA), used to exclude contamination during DNA extraction. Note

1.For samples whose final products are not easily digested by enzymes (proteinase K, trypsin, etc.) due to the use of chemical cross-linking and other

5.3.2 Black 96-well plate.

5.3.3 Fluorescence microplate reader.

5.3.4 Low-adhesion DNase-free sterile centrifuge tube.

5.3.5 Low-retention sterile pipette tip.

5.3.6 Refrigerated centrifuge.

5.3.7 Vacuum dryer.

5.3.8 Oscillator.

5.3.9 Vortex mixer.

6.1 Sample digestion/homogenization

6.1.1 Preparation of test samples and reaction solutions The test samples and reaction solutions are prepared as follows:

a) Before sample testing, it is advisable to prepare parallel samples and determine their moisture content (%) using an appropriate method to calculate the dry weight of the sample.

b) Accurately weigh the test sample (e.g., 5 mg ~ 10 mg) and record the amount; place the sample into a

1.5 mL DNase-free sterile centrifuge tube. Take three parallel samples as the test sample group, and another three parallel samples as the purified spiked and recovered sample group. The different samples are processed as follows: 1) Dry sample. Take the sample and weigh it accurately and record it for subsequent testing. 2) Wet sample. Place the sample in a

1.5 mL DNase-free sterile centrifuge tube and centrifuge at 15 000 g for 10 min; remove the liquid portion; accurately weigh, and record the weight for subsequent testing. 3) Colloidal sample. After freeze-drying, accurately weigh and record the sample; place it in a

1.5 mL DNase-free sterile centrifuge tube. If the addition of lysis buffer during subsequent DNA purification can decompose Take 125 µL of the diluted standard solution per well and add it to a 96-well black ELISA plate, with 3 replicate wells for each sample.

c) Preparation of test samples. Take the purified spiked recovery test DNA sample and the purified test sample DNA sample; add 1x TE buffer to dilute appropriately to obtain 400 µL of dilution solution; add 125 µL to each well of a 96-well black ELISA plate, with 3 replicate wells for each sample.

Note. The dilution factor of the spiked recovery test DNA purification sample and the test sample DNA purification sample can be adjusted. It is advisable to ensure that the fluorescence intensity value after the reaction is within the range of the fluorescence intensity value after the reaction of the standard solution (less than the fluorescence intensity value after the reaction of 80 ng/mL).

d) Add 125 μL of PicoGreen reaction solution to each sample above (mixed with an equal volume of the sample); shake well; incubate at room temperature in the dark for 5 minutes; measure the fluorescence using a fluorescence microplate reader. Determination conditions. Use 480 nm as the excitation wavelength and 520 nm as the emission wavelength for measurement; plot the obtained data and analyze to obtain the regression equation. Use the fluorescence intensity value measured with 1x TE buffer as the sample as the background; measure and record the relative fluorescence intensity (RFI) value of each measurement well. Note

7 Result calculation

7.1 Data processing First, measure the standard and the sample to be tested 5 times to obtain the relative fluorescence intensity value; take the average of the relative fluorescence intensity (RFI) values of the 5 measurements; subtract the background (the RFI value measured when only TE is present and no DNA is present).