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

GB/T 21769-2025

Replacing GB/T 21769-2008

Chemicals In vitro 3T3 NRU phototoxicity test method

化学品 体外3T3中性红摄取光毒性试验方法

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1. Structure and Drafting Rules of Standardization Documents". Drafting. This document supersedes GB/T 21769-2008 "In vitro 3T3 neutral red uptake phototoxicity test method for chemicals", and is consistent with GB/T 21769-2008. In comparison, aside from structural adjustments and editorial changes, the main technical changes are as follows:

a) The phrase "Basic Principles of Experiment" was changed to "Experimental Principles," and the requirements regarding the light treatment time during the experiment were modified (see Chapter 5). Chapter 4 of the 2008 edition);

b) The value of the molar extinction coefficient has been changed (see 6.1.1, 5.1.1 in the 2008 edition);

c) The term "metabolic system" was changed to "metabolic activation system," thus altering the requirements for the metabolic system in the experiment (see 6.1.3, 2008). Version 5.1.3);

d) Added requirements for the selection of experimental cells in "Experimental Preparation" (see 6.2.1) and content on adjusting the carbon dioxide concentration in culture conditions (see [link to relevant section]). 6.2.2) Preparation of a series of diluted solutions of the test substance (see 6.2.4), the full-spectral wavelength range of the light source and its UVA illuminance. The requirements for periodic calibration of the instrument are detailed in section 6.2.5.

e) The section on cell line source in experimental preparation has been removed (see 5.2.1 in the 2008 edition). The section on the selection of weak [cell lines] in the preparation of test chemicals has been revised. The requirements for cell carbon dioxide culture conditions in the negative control group and the contents of the negative control group (see 5.2.4 of the 2008 edition) are as follows: negative... The photosensitivity test results for the control group (see section

5.4.2 of the.2008 edition);

f) Added information regarding the maximum concentration of the test substance being reduced to 100 µg/mL (see 6.3.1.2);

g) Change "Negative Control Group" to "Solvent Control Group" and modify the absolute optical density values (see 6.4.3, see.2008 edition). 5.4.3);

h) Added content on "avoiding crystallization of neutral red solution" (see 6.4.4);

1 Scope

GB/T 21769-2025 is the Chinese national standard on chemicals *in vitro* 3T3 nru phototoxicity test method, in the field of environment and health protection, safety. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 5 October 2025 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 February 2026. Classification: ICS 13.300, CCS A80. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This document establishes the principle of the *in vitro* 3T3 neutral red uptake phototoxicity test for chemicals and specifies the content of the test results and test report. The requirements describe the experimental methods. This document applies to the screening and testing of phototoxicity of chemicals.

2 Normative references

The contents of the following documents, through normative references within the text, constitute essential provisions of this document. Dated citations are not included. For references to documents, only the version corresponding to that date applies to this document; for undated references, the latest version (including all amendments) applies. This document.

GB/T 6682 Specifications and test methods for water used in analytical laboratories

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 irradiance The intensity of ultraviolet (UV) or visible (Vis) light incident on a surface.

Note. The unit is watts per square meter (W/m²) or milliwatts per square centimeter

(mW/cm²).

3.2 Light dose The amount of ultraviolet or visible light incident on a surface (the product of intensity and time).

Note. The number of joules is expressed per unit surface area, i.e., joules per square meter (J/m²) or joules per square centimeter (J/cm²).

3.3 In biological experiments, ultraviolet light has a wavelength of 100nm to 400nm. UVB (280nm~315nm) and UVC (100nm~280nm). The boundary between UVB and UVA is usually at 320nm; UVA can be found at... At 340nm, it is divided into UV-A1 and UV-A2.

3.4 Cellular activity celviability The value of a parameter used to measure the total activity of a cell population (such as the uptake of the active dye Neutral Red by lysosomes) depends on the endpoint of the measurement and The experimental design used, and its correlation with total cell count and/or cell viability.

3.5 The test substance was subjected to both light-free (-Irr) and light-exposed conditions [Irr, non-cytotoxic ultraviolet/visible light (UV/Vis) irradiation], respectively.