Irish Standard
I.S. EN ISO 29701:2010

Nanotechnologies - Endotoxin test on nanomaterial samples for in vitro systems - Limulus amebocyte lysate (LAL) test (ISO 29701:2010)

I.S. EN ISO 29701:2010

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English Version

Nanotechnologies - Endotoxin test on nanomaterial samples for in vitro systems - Limulus amebocyte lysate (LAL) test (ISO 29701:2010)

Nanotechnologies - Essai de détection d'endotoxines sur des échantillons de nanomatériaux pour des systèmes in vitro - Essai au lysat d'amébocyte de Limule (LAL) (ISO 29701:2010)

Nanotechnologien - Endotoxinprüfung an Proben aus nanomaterial für In-vitro-Systeme - Limulus-Amoebozyten-Lysat-Prüfung (LAL-Prüfung) (ISO 29701:2010)

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Foreword

The text of ISO 29701:2010 has been prepared by Technical Committee ISO/TC 229 “Nanotechnologies” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 29701:2010 by Technical Committee CEN/TC 352 “Nanotechnologies” the secretariat of which is held by BSI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2011, and conflicting national standards shall be withdrawn at the latest by March 2011.

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Endorsement notice

The text of ISO 29701:2010 has been approved by CEN as a EN ISO 29701:2010 without any modification.

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I.S. EN ISO 29701:2010

INTERNATIONAL STANDARD

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Nanotechnologies — Endotoxin test on nanomaterial samples for *in vitro* systems — *Limulus* amebocyte lysate (LAL) test

Nanotechnologies — Essai de détection d'endotoxines sur des échantillons de nanomatériaux pour des systèmes in vitro *— Essai au lysat d'amébocyte de* Limule *(LAL)*

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Foreword

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ISO 29701 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

Endotoxins (lipopolysaccharides LPS) are part of the outer membrane of the cell wall of Gram-negative bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus*. Endotoxins can cause a variety of systemic reactions in mammals, including humans, such as fever, disseminated intravascular coagulation, hypotension, shock and death: the responses are mediated by production of various kinds of cytokines, activation of the complement cascade, activation of the coagulation cascade, etc. Endotoxins are present in the ordinary environment. Since most test samples of nanomaterials intended for *in vitro* and *in vivo* test systems require various preparation procedures, endotoxins might contaminate the test nanomaterials if the samples are prepared without special care.

For the purpose of toxicity screening or biocompatibility testing of nanomaterials, or mechanism studies on the possible toxicity induced by nanomaterials, various cell-based *in vitro* test systems and *in vivo* animal models are being developed and employed. In *in vitro* test systems, macrophages and other relevant mammalian cells are frequently used as the test cells especially for nanomaterials because they are primarily the responsible surveillance cells in the body. However, these cells are highly reactive to endotoxins; therefore it is difficult to distinguish the response to endotoxins from that to nanomaterials. Consequently, contamination by endotoxins would confound the result of tests *in vitro*.

Contamination by endotoxins of test samples may be reduced if appropriate precautions are followed in preparation of the test sample. Therefore the preliminary detection of endotoxins is required to minimize the contamination by endotoxins or confirm the insignificant levels of endotoxins in the test sample. It is also important to quantify endotoxin levels for the adequate interpretation of data obtained by *in vitro* biological test systems.

Since endotoxins may contaminate medical devices and medicines for parenteral use, quantitative and semi-quantitative assay methods to test for endotoxins both *in vivo* and *in vitro* have been developed and used for regulatory purposes as well as laboratory standard operational procedures for nanomaterials (see Reference [6]). The bacterial endotoxin test using *Limulus* amebocyte lysate (LAL) reagent has been developed as an *in vitro* assay method to test for the presence of endotoxin contamination as an alternative to the pyrogenicity test using rabbits, and methods are described in the pharmacopoeia of many countries.

This International Standard provides considerations for the application of the LAL test to nanomaterial samples intended for *in vitro* biological tests.