

---

---

**Nanotechnologies — Magnetic  
nanomaterials —**

**Part 2:  
Specification of characteristics  
and measurement methods for  
nanostructured magnetic beads for  
nucleic acid extraction**

*Nanotechnologies — Nanomatériaux magnétiques —*

*Partie 2: Spécification des caractéristiques et des méthodes de mesure  
pour les billes magnétiques nanostructurées pour l'extraction d'acide  
nucléique*



This document is a preview generated by EUS



**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2021

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

	Page
Foreword.....	iv
Introduction.....	v
<b>1 Scope.....</b>	<b>1</b>
<b>2 Normative references.....</b>	<b>1</b>
<b>3 Terms and definitions.....</b>	<b>1</b>
<b>4 Abbreviations.....</b>	<b>3</b>
<b>5 Characteristics to be measured and measurement methods.....</b>	<b>4</b>
5.1 General.....	4
5.2 Descriptions of characteristics and their measurement methods.....	5
5.2.1 Bead mass concentration.....	5
5.2.2 Bead size distribution.....	5
5.2.3 Nucleic acid binding capacity.....	5
5.2.4 Remanent mass magnetization.....	6
5.2.5 Surface functional group type.....	7
5.2.6 Saturation mass magnetization.....	7
5.2.7 Initial magnetic mass susceptibility.....	8
5.2.8 Iron ion concentration.....	8
5.2.9 Mass specific surface area.....	8
5.2.10 Size of primary magnetic nanoparticles.....	9
5.2.11 Surface functional group density.....	9
<b>6 Sample preparation.....</b>	<b>10</b>
<b>7 Test report.....</b>	<b>10</b>
<b>Annex A (informative) Schematics of magnetic beads.....</b>	<b>11</b>
<b>Bibliography.....</b>	<b>12</b>

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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

A list of all parts in the ISO/TS 19807 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

Magnetic beads are composed of a large number of magnetic nanoparticles immobilized within a nonmagnetic matrix with a size range between tens of nanometres and hundreds of micrometres (see [Annex A](#)). The immobilization matrix is typically based on silica or organic polymers. The beads are commonly supplied while dispersed in a liquid suspension, for example, ethanol, isopropanol, sodium azide solutions, pure water. Magnetic beads in liquid suspension have become one of the most widely used nanomaterials in the biological and chemical fields, due to their unique magnetic properties and interactions with applied magnetic fields.

When the size of a magnetic object is small enough, it will form a single magnetic domain, behaving as a single large macrospin. At yet smaller sizes (for iron oxide, typically less than 30nm<sup>[1]</sup>), the thermal energy of the object can be sufficient to result in frequent reorientations of the magnetization direction of the object. If the timescale of these reorientations is shorter than the timescale of the measurement, the term ‘superparamagnetism’ is used to describe this behaviour and the magnetic nano-objects are said to be superparamagnetic. In large non-interacting ensembles of such particles, the thermally induced switching events will result in the average magnetization of the ensembles being zero in the absence of an applied magnetic field. In the presence of an applied large field, the ensemble of magnetic nano-objects is observed to acquire a large net magnetization, as the magnetic field overcomes the thermal fluctuations and aligns the macrospins of the individual magnetic nano-objects within the ensemble. Beads, if incorporating a large fraction of magnetic nano-objects which exhibit this behaviour, are often referred to as “superparamagnetic beads”. However, as the beads may not themselves be superparamagnetic, they are referred to as “magnetic beads” herein.

Magnetic beads have been applied in many fields, especially in biosensing applications<sup>[2]</sup> such as *in vitro* diagnostics, targeted drug delivery<sup>[3]-[5]</sup>, magnetic resonance imaging<sup>[6]</sup>, bioseparation<sup>[7]</sup>, and genetic engineering<sup>[8]</sup>, among others. For example, nucleic acids, which carry genetic information, can be extracted or isolated from blood, saliva, faeces, urine, leaves, viral lysates, using suitably functionalized magnetic beads.

The nucleic acids (DNA) and ribonucleic acid (RNA) carry the key information that organisms use to build or maintain their biostructures. Correctly identifying DNA offers immensely valuable information on health. In recent years, in the human blood stream, scientists have not only found circulating cell free DNA (cfDNA), but also circulating tumour DNA (ctDNA). Now ctDNA extraction is one of the most widely used liquid-biopsy methods to determine cancer or track cancer development. However, the content of ctDNA is only 1 % or less of the total cfDNA amount. The concentration of cfDNA is very low, generally 5 ng/ml blood to 30 ng/ml blood. Therefore, the development of reliable methods for extracting the ctDNA is critical. The proper description of physicochemical characteristics of magnetic beads for DNA extraction is both valuable for developers of extraction kits and for users applying them for DNA analysis.

Nucleic acid binding to magnetic beads relies on electrostatic interactions, hydrophobic interactions, hydrogen bonding or specific binding mechanisms to the bead surface. Once DNA or RNA from cell or tissue lysate is released into the solution, then nucleic acids can bind to surface-modified magnetic beads to form a “nucleic acid-magnetic bead complex”.<sup>[9]-[19]</sup>

Then, the complex can be separated under a proper combination of magnetic field and magnetic field gradient. The eluate can wash away the residual impurities. Finally, the nucleic acids to be extracted can be obtained from the beads after desalination and purification.<sup>[9]-[19]</sup>

The different forms of magnetic beads and dispersing media for the extraction of nucleic acid will have different physicochemical characteristics such as specific surface area, bead concentration etc. All these characteristics will affect their performance to extract nucleic acid to varying extents.<sup>[9]-[19]</sup>

In common with other nanostructured materials, the manufacturing and material specification of composite magnetic beads are complex. Small variations in the synthesis conditions during bead manufacturing and functionalization can lead into dramatic shifts in the properties and binding capacities of the manufactured beads. This requires these products to have high manufacturing consistency. Currently, different manufacturers provide different characteristics and most of them

never provide the measurement methods, so it is difficult for consumers or regulators to compare different products or to verify the characteristics, which increases the difficulty of further development of the application. Universally accepted material specification and test reports for magnetic beads are a requirement in order to ensure customer confidence and the quality of the nucleic acid extraction products.

This document is a preview generated by EVS

# Nanotechnologies — Magnetic nanomaterials —

## Part 2:

## Specification of characteristics and measurement methods for nanostructured magnetic beads for nucleic acid extraction

### 1 Scope

This document specifies characteristics to be measured of magnetic beads in suspension and powder forms for nucleic acid extraction applications. This document deals with magnetic beads that contain a substantial amount of magnetic nanoparticles (which can be superparamagnetic). Potential applicable measurement methods are listed for the individual characteristics. Specifically, this document lists critical characteristics of magnetic beads and suspensions, and additional characteristics to describe the magnetic beads and the suspension for nucleic acid extraction.

Health, safety and environmental aspects of magnetic beads are not within the scope of this document.

### 2 Normative references

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

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

### 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1, ISO/TS 80004-6 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

#### 3.1

##### **bead mass concentration**

ratio of the mass of *magnetic beads* (3.6) to the total volume of a magnetic beads sample in suspension or powder form

#### 3.2

##### **bead size**

effective outer diameter of a *magnetic bead* (3.6) determined by using the specified measurement method