INTERNATIONAL STANDARD

ISO 8036

Redline version
compares Second edition to
First edition

Microscopes — Immersion liquids for light microscopy

Microscopes — Liquides d'immersion pour microscopie optique

Reference number
ISO 8036:redline:2015(E)

IMPORTANT — PLEASE NOTE

This is a mark-up copy and uses the following colour coding:

Text example 1 — indicates added text (in green)

~~Text example 2~~ — indicates removed text (in red)

 — indicates added graphic figure

 — indicates removed graphic figure

1.x ... — Heading numbers containg modifications are highlighted in yellow in the Table of Contents

All changes in this document have yet to reach concensus by vote and as such should only be used internally for review purposes.

DISCLAIMER

This Redline version provides you with a quick and easy way to compare the main changes between this edition of the standard and its previous edition. It doesn't capture all single changes such as punctuation but highlights the modifications providing customers with the most valuable information. Therefore it is important to note that this Redline version is not the official ISO standard and that the users must consult with the clean version of the standard, which is the official standard, for implementation purposes.

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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.

~~International Standards are~~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 ~~rules given in~~editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

~~The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.~~

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).

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 WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary information.

~~ISO 8036 was prepared by Technical Committee~~The committee responsible for this document is ISO/TC 172, *Optics and photonics*, ~~Subcommittee~~ SC 5, *Microscopes and endoscopes*.

~~It~~This second edition cancels and replaces the first edition (ISO 8036~~-1:1998~~:2006~~, which has been technically revised~~), where in Clause 6, the outdated reference to the EU Directive 91/155/EEC and its Amendments 93/112/EC and 01/58/EC were removed.

~~The scope has been extended to include not only immersion oils for general use in light microscopy, but also immersion liquids for use in fluorescence microscopy.~~

Microscopes — Immersion liquids for light microscopy

1 Scope

This International Standard describes the characteristics of immersion liquids used in microscopy. It classifies immersion liquids according to their field of application and specifies requirements and test methods for each type.

This International Standard further specifies a system of designation for immersion liquids, the information to be included on container labels, and the information to be supplied in technical data sheets.

2 Normative references

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

ISO 2592, *Determination of flash and fire points — Cleveland open cup method*

ISO 8255-1, ~~*Optics and optical instruments —*~~ *Microscopes — Cover glasses — Part 1: Dimensional tolerances, thickness and optical properties*

ISO 8255-2, ~~*Optics and optical instruments —*~~ *Microscopes — Cover glasses — Part 2: Quality of materials, standards of finish and mode of packaging*

3 Classification

Depending on their field of application, immersion liquids are classified as follows:

— type N: immersion oil for general use in light microscopy;

— type F: immersion oil which meets the requirements of fluorescence microscopy;

— type G: spectrally pure glycerol (commonly known as glycerine) for glycerol immersion.

4 Characteristics of immersion liquids

4.1 Optical properties

The optical properties of immersion liquids are defined by the refractive index at the wavelength $\lambda = 546{,}07$ nm, n_e, at a defined temperature (23 °C) and pressure (1 013,25 hPa), as well as by the Abbe number (reciprocal of the dispersive power), ν_e.

The Abbe number, ν_e, is calculated using ~~the following equation~~Formula (1):

$$\nu_e = \frac{n_e - 1}{n_{F'} - n_{C'}}$$

$$\nu_e = \frac{n_e - 1}{n_{F'} - n_{C'}}$$

~~where~~

$n_{F'}$	is the refractive index at λ = 479,99 nm;
$n_{C'}$	is the refractive index at λ = 643,85 nm.

where

$n_{F'}$ is the refractive index at λ = 479,99 nm;

$n_{C'}$ is the refractive index at λ = 643,85 nm.

The refractive indices are usually measured with an Abbe refractometer at constant temperature with a tolerance of ±0,1 °C. A mercury-cadmium spectral lamp is used as the light source.

4.2 Transmittance

Modern detection methods in light microscopy require an extension of the spectral range of immersion liquids.

The transmittance is measured using a suitable spectrophotometer in quartz cuvettes with an optical path length through the immersion liquid of d = 10 mm against an empty reference cuvette.

4.3 Autofluorescence

The autofluorescence of immersion liquids is measured with a fluorescence spectrometer. Quinine sulfate in 0,05 mol/l sulfuric acid is used as the fluorescence standard. For the fluorescence measurement, the following excitation and emission wavelengths are used:

— F(365 nm/450 nm) = fluorescence excitation at 365 nm, measurement of fluorescence emission at 450 nm;

— F(405 nm/485 nm) = fluorescence excitation at 405 nm, measurement of fluorescence emission at 485 nm.

As the fluorescence emission decreases during the measurement, the autofluorescence of immersion liquids is averaged over a measurement time of 60 s.

Immersion liquid samples and standard liquids are measured in a fluorescence cuvette made of quartz or special optical glass with an optical path length through the immersion liquid of d = 10 mm. The reagents used shall meet the quality standards for fluorescence spectroscopy.

Prepare a quinine sulfate stock solution of 500 mg/l with 0,05 mol/l sulfuric acid. Before use, prepare two standard solutions of 0,1 mg/l and 2,5 mg/l quinine sulfate in 0,05 mol/l sulfuric acid by diluting the stock solution 1:5000 and 1:200, respectively. Store the stock solution in a closed brown glass bottle at 6 °C to 12 °C for no more than two months. Always prepare the standard solutions fresh on the day of use.

As an alternative to quinine sulfate solution, solid-state fluorescence standards with the same shape as a 10 mm cuvette may be used. These shall be calibrated against a quinine sulfate standard solution at least once a year.

NOTE 1 Solid-state fluorescence standards are available as spectroscopy accessories.

The information regarding the autofluorescence of the immersion liquids is given in terms of the equivalent amount of quinine sulfate, in mg/l.

NOTE 2 Quinine sulfate is more suitable for use as a fluorescence standard than rhodamine B because immersion oils tend, like quinine sulfate, to emit in the blue region in the case of UVA excitation, while rhodamine B emits in the green region.

4.4 Viscosity

The viscosity of the immersion liquid shall be measured by means of a capillary viscometer, e.g. an ~~Ubbelhode~~Ubbelohde viscometer, at a temperature of 23 °C.

4.5 Density

The density of the immersion liquid shall be measured at a temperature of 23 °C by means of a hydrometer or a pyknometer.

5 Minimum requirements

See Table 1.

6 Ingredients

Immersion liquids conforming to this International Standard shall not contain ingredients which are known to have any adverse effects on safety or health.

~~The manufacturer shall provide the user with a safety data sheet in accordance with EU Directive 91/155/EEC and its Amendments 93/112/EC and 01/58/EC.~~

7 Designation of immersion liquids

Immersion liquids conforming to this International Standard shall be designated as in the following example:

EXAMPLE Immersion liquid ISO 8036~~ Type F~~ Type F.

8 Labelling of containers

Immersion liquids are commonly packed and marketed in brown~~-~~ glass bottles or in oilers made from polyethylene or polypropylene. The labelling of these containers shall include the following information:

— the product name;

— the designation in accordance with this International Standard;

— labelling required by regulations for hazardous freight;

— the refractive index, n_e, at 23 °C;

— the Abbe number, v_e;

— the batch number;

— the expiration date when stored in the unopened original packaging in accordance with the ~~manufacturer's~~manufacturer's instructions;

— information on how to obtain technical and safety data;

— the name and address of the manufacturer and the supplier, plus the country of origin if different from that given in the manufacturer's address.

~~**Table 1 — Minimum requirements for immersion liquids**[a]~~

Table 1 *(continued)*

Parameter		**Type N (standard)**	**Type F (for fluorescence)**	**Type G (glycerol)**[b]
Reference temperature (°C)		23	23	23
Tolerance (°C)		± 0,1	± 0,1	± 0,1
Spectral range (nm)		400 to 900	320 to 1 100	230 to 1 100
Refractive index, n_e		1,518 0 ± 0,000 5	1,518 0 ± 0,000 5	1,450 ± 0,005
Abbe number, v_e		43 ± 4	43 ± 4	58 ± 4
Transmittance (d = 10 mm)				
	at 1 100 nm	—	80 %	70 %
	at 1 000 nm	—	80 %	70 %
	at 900 nm	90 %	90 %	90 %
	at 800 nm	95 %	95 %	98 %
	at 760 nm	95 %	95 %	98 %
	at 600 nm	95 %	95 %	98 %
	at 500 nm	95 %	95 %	98 %
	at 450 nm	85 %	95 %	98 %
	at 420 nm	75 %	95 %	98 %
	at 400 nm	60 %	95 %	98 %
	at 380 nm	—	90 %	95 %
	at 365 nm	—	80 %	95 %
	at 350 nm	—	70 %	90 %
	at 330 nm	—	40 %	80 %
	at 320 nm	—	20 %	70 %
	at 310 nm	—	—	50 %
	at 230 nm to 300 nm	—	—	30 %
Autofluorescence (mg/l quinine sulfate)				
	F(365 nm/450 nm)	[c]	0,06	0,02
	F(405 nm/485 nm)	[c]	1,20	0,50
	F(313 nm/450 nm)	—	—	0,05
Viscosity (mm^2/s)				
	at 23 °C ± 0,1 °C	50 to 1 500	50 to 1 500	[b]

[a] Immersion liquids, with the exception of glycerol, may not contain volatile or hygroscopic ingredients. Specifications for these liquids assume the use of a cover glass as specified in ISO 8255-1 and ISO 8255-2.

[b] Glycerol is hygroscopic and its physical properties depend on its water content.

[c] Autofluorescence of type N oil has no specified values, but high values can be expected.

Table 1 — Minimum requirements for immersion liquids[aa]

Table 1 *(continued)*

Parameter	Type N (standard)	Type F (for fluorescence)	Type G (glycerol)[b]
Reference temperature (°C)	23	23	23
Tolerance (°C)	±0,1	±0,1	±0,1
Spectral range (nm)	400 to 900	320 to 1 100	230 to 1 100
Refractive index, n_e	1,518 0 ± 0,000 5	1,518 0 ± 0,000 5	1,450 ± 0,005
Abbe number, ν_e	43 ± 4	43 ± 4	58 ± 4
Transmittance (d = 10 mm)			
at 1 100 nm	—	80 %	70 %
at 1 000 nm	—	80 %	70 %
at 900 nm	90 %	90 %	90 %
at 800 nm	95 %	95 %	98 %
at 760 nm	95 %	95 %	98 %
at 600 nm	95 %	95 %	98 %
at 500 nm	95 %	95 %	98 %
at 450 nm	85 %	95 %	98 %
at 420 nm	75 %	95 %	98 %
at 400 nm	60 %	95 %	98 %
at 380 nm		90 %	95 %
at 365 nm	—	80 %	95 %
at 350 nm	—	70 %	90 %
at 330 nm	—	40 %	80 %
at 320 nm	—	20 %	70 %
at 310 nm	—	—	50 %
at 230 nm to 300 nm	—	—	30 %
Autofluorescence (mg/l quinine sulfate)			
F(365 nm/450 nm)	[c]	0,06	0,02
F(405 nm/485 nm)	[c]	1,20	0,50
F(313 nm/450 nm)	—	—	0,05
Viscosity (mm²/s)			
at 23 °C ± 0,1 °C	50 to 1 500	50 to 1 500	[b]

[a] Immersion liquids, with the exception of glycerol, may not contain volatile or hygroscopic ingredients. Specifications for these liquids assume the use of a cover glass as specified in ISO 8255-1 and ISO 8255-2.

[b] Glycerol is hygroscopic and its physical properties depend on its water content.

[c] Autofluorescence of type N oil has no specified values, but high values can be expected.

9 Technical data sheet

The manufacturer or the supplier of an immersion liquid shall make a technical data sheet available on request. This data sheet shall contain the following information:

— product name;

— manufacturer;