DaraEx

These instructions for use are valid for all current batches of DaraEx with purple lids.

Version 22_EN_RUO, 2024-07-01

Σ	Up to 30 tests per sales unit (300 µl)
2°C 8°C	Store at 2...8°C

1. Introduction

1.1. Overview

This manual describes the use of imusyn's

anti-CD38 antibody neutralizing agent (DaraEx)

to inhibit the agglutination effect of the anti-CD38 antibodies Daratumumab, Felzartamab, Isatuximab and Mezagitamab in the indirect antiglobulin test (IAT).

Anti-CD38 antibodies can interfere with the crossmatch, the antibody search, and the antibody identification in the IAT. This interference can occur up to 6 months after the last administration of the drug [1].

1.2. Test Principle

DaraEx masks CD38 on the surface of red blood cells, thereby preventing the anti-CD38 antibodies Daratumumab, Felzartamab, Isatuximab and Mezagitamab from binding and inducing agglutination.

1.3. Intended Purpose

For research use only.

2. Materials and Equipment

2.1. Components

DaraEx DaraEx 300 µl per sales unit, conserved with 0.1% ProClin® 300

WARNING!

May cause an allergic skin reaction (H317). Harmful to aquatic life with long lasting effects (H412). Wear protective gloves (P280). If skin irritation or rash occurs: Get medical advice/attention (P333+P313). Dispose of contents/container in accordance with local/regional/national/international regulations (P501).

Safety Data Sheet (SDS) available on imusyn.de/IFU.

2.2. Storage, Expiry Date, and Disposal

Store at 2…8°C. If the storage conditions are met, DaraEx can be used until the expiration date given on the label and the certificate of analysis. DaraEx and its containers must be disposed of properly according to local guidelines.

⚠ Do not freeze DaraEx! The reactivity of frozen or frozen and thawed DaraEx cannot be guaranteed. The according container has to be disposed of immediately!

2.3. Materials and Equipment Supplied by the User

Materials and Equipment	Supplier
- ID-Card LISS/Coombs - Test cell preparations for the ID System	Bio-Rad
Alternatively - MTS™ Anti-IgG Card - Test cell preparations for the MTS System	Ortho Clinical Diagnostics
- Process control PC e.g. Dara-PC, or 0.5 mg/ml daratumumab in NaCl, or a known and otherwise non-reactive daratumumab-containing specimen	Not applicable / imusyn
If applicable - Reaction vessels, PP	Multiple suppliers
If applicable - NaCl	Multiple suppliers
- Centrifuge for cards or work station, matching the card system used	Bio-Rad / Ortho Clinical Diagnostics
- Incubator, 37°C	Multiple suppliers
- Pipettes and pipette tips	Multiple suppliers
- Tabletop centrifuge	Multiple suppliers

Note: All materials and devices indicated with a specific manufacturer have been tested for use with DaraEx.

3. Preparation and Usage

During all activities, care must be taken to avoid contaminations. The reagents used must be brought to room temperature before use. The card manufacturer's requirements must also be observed.

⚠ DaraEx is a clear and colorless solution. Do not use DaraEx if its color has changed or if the solution is clouded!

Only use DaraEx in undamaged primary packaging! Damaged DaraEx containers must be disposed of.

3.1. Express Protocol

Use this protocol for 0.8% red blood cell preparations (e.g. test cell panels or preparations prepared from a red blood cell concentrate).

3.1.1. Test Cell Treatment

To 1 volume of red blood cells (0.8%), add 0.2 volumes of DaraEx, e.g. to 50 µl of cells add 10 µl of DaraEx. The cells can be used immediately; the addition can be done directly in the card or in a separate reaction vessel. Incubation in advance is not necessary.

⚠ The test cell concentration is critical! Cells that are concentrated above 0.8% need higher volumes of DaraEx added (see section 3.2)!

3.1.2. Test Procedure

Use the DaraEx-treated cells in the IAT system according to the manufacturer's instructions for use.

In addition to the specimens, a process control PC should be included. If a cell is still agglutinated by a specimen after DaraEx treatment, it is mandatory to test the affected cell with the PC or to repeat the test with the specimen in the alternative protocol (section 3.2). Use the PC like a specimen. The test with the PC is successful if no agglutination with DaraEx-treated cells occurs.

⚠ The sequence of pipetting is a critical factor! The treatment of the cells with DaraEx (section 3.1.1) must take place before addition of the specimen or PC to the IAT (section 3.1.2)!

3.2. Alternative Protocol

Use this protocol directly or if the express protocol did not provide satisfactory results. It is only applicable with 1.6% red blood cell preparations.

3.2.1. Test Cell Preparation

Prepare 1.6% red blood cells according to local instructions for the preparation of red blood cells. For example, centrifuge 50 µl of 0.8% red blood cells for 5 min at 1,000xg and remove 25 µl of the supernatant. The resuspended cells have a concentration of 1.6%.

3.2.2. Test Cell Treatment

To 1 volume of red blood cells (1.6%), add the same volume of DaraEx (final cell concentration 0.8%), e.g. to 25 µl cells add 25 µl of DaraEx. The cells can be used immediately; the addition can be done directly in the card or in a separate reaction vessel. Incubation in advance is not necessary.

3.2.3. Test Procedure

Use the DaraEx-treated cells in the IAT system according to the manufacturer's instructions for use.

In addition to the specimens, a process control PC should be included. If a cell is still agglutinated by a specimen after DaraEx treatment, it is mandatory to test the affected cell with the PC. Use the PC like a specimen. The test with the PC is successful if no agglutination with DaraEx-treated cells occurs.

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⚠ The sequence of pipetting is a critical factor! The treatment of the cells with DaraEx (section 3.2.2) must take place before addition of the specimen or PC in the IAT (section 3.2.3)!

4. Analysis and Troubleshooting

4.1. Analysis

Treatment of the test cells with DaraEx should in most cases completely inhibit the agglutination caused by anti-CD38 antibodies. The IAT can be evaluated as if no anti-CD38 antibody was present in the specimen.

DaraEx-treated cells should not react with PC. If the cells agglutinate with both the PC and the specimen, the test result is invalid and cannot be used.

4.2. Troubleshooting

Problem	Possible Cause	Solution
DaraEx-treated cells are agglutinated by the specimen, but not by the PC.	Incomplete inhibition of agglutination mediated by therapeutic anti-CD38 antibodies.	If the procedure was performed according to section 3.1, adjust the test cell concentration to 1.6% and repeat the test according to section 3.2.
	Irregular antibodies in the specimen.	Evaluate the IAT as if no anti-CD38 antibody was present in the specimen (section 4.1).
	Anti-CD38 antibody concentration in the specimen is too high.	See section 5.1 Limitations.
DaraEx-treated cells are agglutinated by both the PC and the specimen.	Wrong sequence of pipetting (addition of DaraEx after or together with the addition of PC or specimen to cells).	Ensure that the PC and specimen are added after the treatment of the cells with DaraEx.
	Incomplete inhibition of agglutination mediated by therapeutic anti-CD38 antibodies.	Ensure that the procedure has been followed according to instructions and repeat the test if necessary.
		If the procedure was performed according to section 3.1, adjust the test cell concentration to 1.6% and repeat the test according to section 3.2.
	CD38 expression on the test cells used is too high.	If possible, repeat the test using other test cells.

5. Limitations and Interfering Substances

5.1. Limitations

DaraEx was tested with the standard volumes used in the indicated card systems. The use of volumes other than those specified in the card manufacturers' instructions for use, especially the use of higher specimen volumes, may lead to incomplete inhibition of anti-CD38 antibody interference. The usage of other card systems or IAT procedures than listed in section 2.3 may cause false results and must thus be validated by the user beforehand.

Specimens from patients with high levels of free anti-CD38 antibody, e.g. patients recently treated with therapeutic anti-CD38 antibodies, or cells with high CD38 expression may not be fully inhibited.

DaraEx has only been tested with respect to inhibition of agglutination by anti-CD38 antibodies listed in section 1.1. Inhibition of other antibodies, including other anti-CD38 antibodies, by DaraEx has not been tested.

Failure to follow these instructions for use may lead to false results. In particular, the use of more cells or cells of a higher concentration may cause incomplete inhibition of anti-CD38 interference. Prolonged incubation of cells with DaraEx, e.g. by storing treated cells, has not been tested and may also lead to false results.

The treatment of the test cells according to section 3.1 leads to a slight dilution of the specimen in the test system (usually around 12%). It cannot be excluded that this may result in a reduction of the reaction strength of low-titer antibodies.

Treatment of test cells with DaraEx may lead to a specific enhancement of agglutination by anti-M or anti-N antibodies by up to one reaction strength.

Some card systems are more sensitive to anti-CD38 antibodies than others. If problems with incompletely inhibited anti-CD38 antibody reactions persist, a change of the card system may help.

Contamination of reagents or specimens, use of reagents beyond their expiration date, and use of non-recommended reagents and equipment may cause false results.

5.2. Interfering Substances

The preservative ProClin® 300 contained in the storage buffer of DaraEx was found not to interfere with the reactions in the IAT.

6. References

1. Oostendorp M, Lammerts van Bueren JJ, Doshi P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. Transfusion. 2015;55(6 Pt 2):1555-1562.

7. Definition of Symbols and Abbreviations

NaCl	0.9% sodium chloride solution
LOT	Batch code
REF	Catalogue number
⚠	Caution
	Consult instructions for use
DaraEx	DaraEx
	Use by date (YYYY-MM)
	Exclamation mark (GHS07) – Warning
RUO	For research use only
	Manufacturer
	Contains sufficient for <n> tests
PC	Process control (see also section 2.3)
	Temperature limit

Patent EP3548898B1.

⚠ Please check imusyn.de/IFU regularly for updates to these instructions for use.

Changes to the previous version are highlighted.

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