DaraEx*plus*

Version 8.0_EN_CE, 2022-09-14

IVD	In vitro diagnostics (IVD)
Σ	~ 30 tests
2°C 8°C	2...8°C
	See package printings

ATTENTION! Any serious incident that has occurred in connection with this IVD shall be reported to the manufacturer and to the competent authority of the member state in which the user and/or the patient are established.

1. Introduction

1.1. Overview

These instructions for use describe the use of imusyn's

anti-CD38 antibody neutralizing agent (DaraEx*plus*)

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

Anti-CD38 antibodies interfere with the crossmatch and antibody search in the IAT, resulting in false positive reactions. For Daratumumab, this interference can occur up to 6 months after the last administration of the drug[1].

1.2. Test Principle

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

1.3. Statement of Intended Use

DaraEx*plus* is a diagnostic aid for eliminating the interference of therapeutic anti-CD38 antibodies (Daratumumab, Isatuximab, and Felzartamab) in the determination of irregular antibodies in the IAT. DaraEx*plus* is intended for manual use with gel card systems. DaraEx*plus* is to be used by qualified personnel only in accordance with current local guidelines and is not intended for use by or on patients. DaraEx*plus* does not provide qualitative, semi-quantitative, or quantitative information about anti-CD38 antibodies in the patient specimen.

2. Materials and Equipment

2.1. Definition of Symbols

DaraEx*plus*	DaraEx*plus*
PC	process control, e.g. Dara-PC
NaCl	0.9% sodium chloride solution

2.2. Components

DaraEx*plus* DaraEx*plus* Fab fragment of an anti-CD38 antibody, 300 µl total volume, protein concentration ≥ 5 mg/ml, 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).

2.3. Storage and Expiry Date

Store at 2…8°C. If the storage conditions are met, DaraEx*plus* can be used until the expiration date given on the primary packaging and the certificate of analysis.

2.4. Materials and Equipment Supplied by the User

Materials and Equipment	Supplier
- ID-Card LISS/Coombs - Test cell preparations for the ID System	Bio-Rad
- Anti-Human Globulin Anti-IgG Polyspecific (Rabbit) MTS Card - Test cell preparations for the MTS System	Ortho Clinical Diagnostics
- PC (e.g. a Daratumumab solution, or Dara-PC)	Not applicable / imusyn
If applicable - Reaction vessels, PP	Multiple suppliers
If applicable - NaCl	Multiple suppliers
- Centrifuge for gel cards or work station, matching the gel 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 validated for use with DaraEx*plus*. Other materials or devices must be validated by the user before use.

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.

WARNING! Only use DaraEx*plus* in undamaged primary packaging! Damaged DaraEx*plus* containers must be disposed of properly according to local guidelines.

3.1. Specimen Preparation

Do not use haemolytic or lipemic serum or plasma specimens. Plasma may be collected using the anticoagulants CPD-A, citrate, or EDTA. Particles, aggregates, or fibrin residues must be removed prior to testing to avoid non-specific results. Red blood cell preparations must have been prepared and stored according to the manufacturers' instructions or local instructions for preparation and storage of red blood cell concentrates. The gel card manufacturer's restrictions on sample material must also be observed.

WARNING! Human specimens are potentially infectious. The specimens must be handled according to local guidelines and the appropriate protective measures must be taken.

3.2. 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).

3.2.1. Test Cell Preparation

To 1 volume of cells (0.8%), add 0.2 volumes of DaraEx*plus*, e.g. to 50 µl of cells add 10 µl of DaraEx*plus*. The cells can be used immediately, the addition can be done directly in the gel card or in a separate reaction vessel.

WARNING! The test cell concentration is critical! Cells that are concentrated above 0.8% need higher volumes of DaraEx*plus* (s. section 3.3)!

3.2.2. Test Procedure

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

In addition to the specimens, a known and otherwise non-reactive anti-CD38 antibody-containing sample or solution must be included as process control PC. The use of Dara-PC or 0.5 mg/ml Daratumumab in NaCl as PC is recommended. Each DaraEx*plus*-treated cell must be incubated with PC at least once to confirm the neutralization of CD38 at the cell surface.

WARNING! The sequence of pipetting is a critical factor! The treatment of the cells with DaraEx*plus* (section 3.2.1) must take place before addition of the specimen or PC to the IAT (section 3.2.2)!

3.3. Alternative Protocol

Use this protocol for 1.6% red blood cell preparations, e.g. if the express protocol was not successful.

3.3.1. Test Cell Preparation

To 1 volume of red blood cells (1.6%), add the same volume of DaraEx*plus* (final cell concentration 0.8%), e.g. to 25 µl cells add 25 µl of DaraEx*plus*. The cells

imusyn GmbH & Co.KG · Feodor-Lynen-Str. 5 · 30625 Hannover, Germany · Tel: +49 511 563585-0 · Fax: +49 511 563585-29 · www.imusyn.de · info@imusyn.de

can be used immediately, the addition can be done directly in the gel card or in a separate reaction vessel.

3.3.2. Test Procedure

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

In addition to the specimens, a process control PC, as described in section 3.2.2, must be included. Each DaraEx*plus*-treated cell must be incubated with PC at least once to confirm the neutralization of CD38 at the cell surface.

⚠ WARNING! The sequence of pipetting is a critical factor! The treatment of the cells with DaraEx*plus* (section 3.3.1) must take place before addition of the specimen or PC in the IAT (section 3.3.2)!

4. Analysis and Troubleshooting

4.1. Analysis

Treatment of the test cells with DaraEx*plus* 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*plus*-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*plus*-treated cells are agglutinated by both the PC and the specimen.	Wrong sequence of pipetting (addition of DaraEx*plus* 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*plus*.
	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 3.2, adjust the test cell concentration to 1.6% and repeat the test according to section 3.3.
	CD38 expression on the test cells used too high.	If possible, repeat the test using other test cells.

Please also observe the instructions of the gel card manufacturer on error handling and the limits of the procedure! For technical support you may also contact the manufacturer (contact see below).

5. Limitations and Specific Characterization

5.1. Limitations

DaraEx*plus* was tested with the standard volumes in the indicated gel card systems. The use of volumes other than those specified in the gel card manufacturers' instructions for use, especially the use of higher specimen volumes, may lead to incomplete inhibition of anti-CD38 antibody interference. The use of gel card systems or IAT methods other than those listed in section 2.4 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*plus* has only been validated 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*plus* has not been tested.

Failure to follow this instruction 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*plus*, e.g. by storing treated cells, may also lead to false results.

The treatment of the test cells according to section 3.2 leads to a slight dilution of the specimen in the test system (usually about 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*plus* may lead to a specific enhancement of agglutination by anti-M or anti-N antibodies by up to one reaction strength.

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*plus* was found not to interfere with the reactions in the IAT.

5.3. Specific Characterization

Clinical performance data show better performance (complete elimination of Daratumumab-mediated interference) of DaraEx*plus* than DTT treatment (as described[3]).

Treatment	Performance	$n_{inhibited}$ / n_{total}
DaraEx*plus*	86.5%*	128 / 148
DTT	68.2%	101 / 148

* For performance evaluation, the tests were first performed according to section 3.2 (express protocol). If inhibition was incomplete (62), the test was repeated according to section 3.3 (alternative protocol). For performance evaluation, tests were counted in which either the express protocol (86/148) or the repetition of the test in the alternative protocol (42/62) completely abolished the anti-CD38 antibody-mediated interference.

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.
2. Chapuy CI, Nicholson RT, Aguad MD, et al. Resolving the daratumumab interference with blood compatibility testing. *Transfusion*. 2015;55(6 Pt 2):1545-1554.
3. Empfehlung zum Vorgehen bei Störungen der serologischen Diagnostik durch Daratumumab und andere therapeutische monoklonale Antikörper gegen CD38, Version 2 vom 01.07.2019, Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie (DGTI), Sektion V: Immunhämatologie und Immungenetik.

Patent EP3548898B1.

⚠ ATTENTION! Please check imusyn.de/IFU regularly for updates to this user manual.

Changes to the previous version are highlighted.

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imusyn GmbH & Co.KG · Feodor-Lynen-Str. 5 · 30625 Hannover, Germany · Tel: +49 511 563585-0 · Fax: +49 511 563585-29 · www.imusyn.de · info@imusyn.de