Anti-CCD Absorbent
Test instruction

ORDER NO.	FORMAT
ZD 3001-0101	01 x 01 (01)
ZD 3001-0401	04 x 01 (04)

Indication: Anti-CCD Absorbent is a supplementary reagent designed for the incubation with EUROIMMUN allergy profiles (product classification DQ, DP, DF, DE). The absorbent is used in case IgE antibodies against cross-reactive carbohydrate determinants (CCDs) were detected in the patient sample (anti-CCD IgE antibodies). The presence of these antibodies is indicated by a positive CCD band on the allergy profiles.

Application: Anti-CCD IgE antibodies can lead to positive results in extract-based in-vitro allergy diagnostics, as they bind to CCDs of glycosylated proteins (e.g. allergens). However, as these anti-CCD IgE antibodies have in general no clinical relevance, their presence complicates the interpretation of positive test results. The Anti-CCD Absorbent binds to the anti-CCD IgE antibodies in the serum and inhibits binding of these antibodies to CCDs of glycosylated allergen extracts. Thus, it impedes positive results that are only based on this binding. Using the Anti-CCD Absorbent therefore increases the specificity of the test results of the allergy profiles when anti-CCD IgE antibodies are present in the patient sample.

Principle of the test: In case anti-CCD IgE antibodies were detected in the patient sample (positive CCD band in EUROIMMUN allergy profiles or findings with other in-vitro test systems), the serum should be re-incubated in a EUROLINE assay using the Anti-CCD Absorbent. For this purpose the Anti-CCD Absorbent is added to the patient sample before the incubation with the allergy profile. The absorbent binds to the anti-CCD IgE antibodies, so that they can no longer bind to the CCD structures of the allergen extracts on the allergy profiles. When using the absorbent, the test instruction of the respective allergy profile (EUROLINE) is to be followed in addition to the details mentioned here.

Contents of the test kit:

Component	Format	Format	Symbol
1. Anti-CCD Absorbent	1 x 40 µg	4 x 40 µg	CCD ABSORBENT
2. Test instruction	1 booklet	1 booklet	

LOT Lot description	CE		Storage temperature
IVD In vitro diagnostic medical device			Unopened usable until

Modifications to the former version are marked in grey.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use.

Anti-CCD Absorbent: The reagent must be dissolved in distilled water (optimal: aqua pro infusione, aqua ad injectabilia) before use. To this end, the lyophilised substance is restored in 110 µl distilled water, mixed for approx. 30 seconds on a vortex and then centrifuged at 17,000 x g (minimum 20 seconds). After reconstitution the reagent has to be stored at +2 °C to +8 °C and protected against contamination. The reconstituted reagent is stable for 4 weeks.

Storage and stability: The undissolved reagent has to be stored at -20°C. Unopened, it is stable until the indicated expiry date. After reconstitution, the reagent can be stored at -20 °C for 6 months and at +2 °C to +8 °C for 4 weeks. For storage of the **reconstituted reagent** at -20 °C, aliquotation is recommended.

Waste disposal: All reagents must be disposed of in accordance with local disposal regulations.

Warning: The reagent contains sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Samples are diluted in accordance to the incubation version given in the test instruction of the allergy profile used.

Pretreatment of the patient sample with Anti-CCD Absorbent

For each sample to be tested, the sample volume is mixed with the respective amount of Anti-CCD Absorbent according to the following overview. Note that different volumes are to be used for manual, semi-automated and automated incubation, respectively. Anti-CCD Absorbent is pipetted into the sample and the preparation is incubated for 60 minutes at room temperature (+18 °C to +25 °C) on a shaker.

Overview of incubation volumes for using the **EUROLINE**:

Manual incubation:

Incubation version	Serum or plasma volume	Anti-CCD Absorbent
Version a (time-optimised)	400 µl	20 µl
Version b (volume-/time-optimised)	175 µl	9 µl
Version c (volume-optimised)	100 µl	5 µl

After that, incubation is continued according to the test instruction of the test system to be incubated. The entire reaction (serum/plasma + Anti-CCD Absorbent) is used for the incubation.

Semi-automated incubation (EUROBlotMaster):

Incubation version	Serum or plasma volume	Anti-CCD Absorbent
Version a (time-optimised)	400 µl	20 µl
Version b (volume-/time-optimised)	210 µl	10.5 µl
Version c (volume-optimised)	150 µl	7.5 µl

After that, incubation is continued according to the details in the test instruction of the test system to be incubated. The entire reaction (serum/plasma + Anti-CCD Absorbent) is used for the incubation.

Automated incubation (EUROBlotOne):
For pre-incubation with the Anti-CCD Absorbent the dead volume of 150 µl is added to the actual incubation volume. The following volumes result:

Incubation version	Serum or plasma volume	Anti-CCD Absorbent
Version a (time-optimised)	550 µl	27.5 µl
Version b (volume-/time-optimised)	360 µl	18 µl
Version c (volume-optimised)	300 µl	15 µl

The reaction tube (serum/plasma + Anti-CCD Absorbent) is transferred to the EUROBlotOne. After that, the allergy profiles are automatically incubated according to the programming of the EUROBlotOne. Here, the volume actually needed for the incubation of the EUROLINE is used (version a: 400 µl, version b: 210 µl, version c: 150 µl).

Evaluation

Evaluation of the test strips (EUROLINE) is carried out according to the instruction of the test system used. After that, the results of the inhibited sample are compared to those of the non-inhibited sample (see chapter “Evaluation with EUROLineScan Software” below). Successful inhibition is indicated by a negative CCD band on the allergy profile used compared to the non-inhibited sample.

a) For those allergen bands that give positive signals in the non-inhibited sample and that are negative in the inhibited sample, the previously obtained positive signal is exclusively caused by anti-CCD IgE antibodies. Most likely, no IgE antibodies which might be of clinical relevance are present against these allergen extracts.
b) Those allergen extracts that show significantly weaker though positive signals compared to the non-inhibited sample contain anti-CCD IgE antibodies as well as IgE antibodies against proteinogenic structures.
c) Those allergen bands that show the same positive results with and without inhibition only contain IgE antibodies against proteinogenic structures.

In the last two cases the clinical relevance of the specific IgE antibodies (antibodies against proteinogenic structures) must be evaluated in relation to the patient’s anamnesis and other diagnostic methods (e.g. skin tests and/or provocation tests).

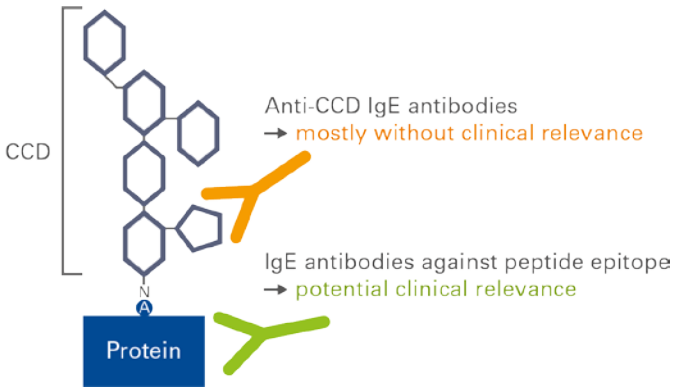
The Anti-CCD Absorbent inhibits IgE antibodies against the most frequent N-glycan structures from plants, insects and shellfish. In rare cases samples with very high concentrations of anti-CCD IgE antibodies (>100kU/l, class 6) can possibly not be completely inhibited and continue to show a positive though significantly weaker CCD band. In these cases increasing the concentration of anti-CCD Absorbent can possibly lead to a complete inhibition. Moreover, using CCD-free allergen components as available on the DPA-Dx Profiles, allows a statement on the sensibilisation independent of CCD based reactions.

Evaluation with EUROLineScan Software

From version 3.4.21 onwards, the EUROLineScan Software, allows to directly compare the results of CCD inhibited and non-inhibited samples. For this, the same EUROLINE Profile must be used for the incubation of the sample with added Anti-CCD Absorbent and for the incubation of the non-inhibited sample. (Attention: Do not use strips that have been incubated before). In addition, the patient ID must be identical to ensure that EUROLineScan Software matches up both strips.
After the incubation, the option “CCD inhibition” in the detailed view can be chosen for one of the strips. In the window that opens, the corresponding strip is selected and the results appear in a table. A comprehensive report of the results can be displayed and/or printed under “detailed report”.

Clinical significance

CCDs are sugar structures which are covalently added to proteins in posttranslational glycosylation. CCD structures of glycosylated proteins of plants and invertebrates differ from those of human glycoproteins and are therefore immunogenic.
At first contact with a glycosylated allergen, specific IgE antibodies against the protein part and against the CCD structures can be generated (anti-CCD IgE antibodies, see figure below). Anti-CCD IgE antibodies can be detected in approx. 25% of patients suffering from allergies, but also in non-allergic individuals.



Due to the high structural similarity of CCDs of different species, antibodies to glycoproteins from insects, shellfish, plant pollen, fruits and latex show cross-reactions.
Moreover, anti-CCD IgE antibodies in general have no clinical relevance. In extract-based in-vitro allergy diagnostics, the presence of anti-CCD IgE antibodies complicates the interpretation of positive results as reactions due to antibodies against peptidic epitopes, anti-CCD IgE antibodies or both cannot be differentiated.
In contrast, CCD interactions are not expected when using recombinantly produced allergen components, because they do not contain any CCD structures due to the selected expression system. Furthermore, CCD-based reactions do not occur against animal allergen sources such as milk and dairy products, eggs, meat and fish, animal hair, feathers and epithelia, moulds, yeasts, and mites.

Literature references

1. Altmann F. **Coping with cross-reactive carbohydrate determinants in allergy diagnosis**. Allergo J Int. 2016; 25:98-105.
2. Mari A. **IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity.** Int Arch Allergy Immunol. 2002, 129 (4): 286-295
3. Holzweber F et al. **Inhibition of IgE binding to cross-reactive carbohydrate determinants enhances diagnostic selectivity.** Allergy. 2013 Oct; 68(10): 1269-1277.
4. Malandain H et al. **The influence of carbohydrate structures present in common allergen sources on specific IgE results.** Eur Ann Allergy Clin Immunol. 2007 Sep;39(7): 216-220.
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6. Aalberse RC, van Ree R. **Crossreactive carbohydrate determinants.** Clin Rev Allergy Immunol. 1997 Winter;15(4): 375-387.