ASCA IgA ELISA

Order Code: ASCA02-96

1. INTENDED USE

The BlueWell ASCA IgA ELISA kit allows the semi-quantitative detection of IgA antibodies to S.cerevisiae in human serum.

This kit is intended to confirm medical observations obtained by intestinal endoscopy and/or results obtained by Indirect Immunofluorescence with smears of baker's yeast, as an aid in the diagnosis of Crohn's disease (for more details, see 11.5 Auto-antibody diagnostic value)..

The test is intended for a large, routine population. This kit is strictly reserved for professional use into clinical analysis laboratories. It can only be used manually or in an open automated ELISA processing system, programmed according to the pipetting scheme described in point 9.2.

2. PRINCIPLE OF THE TEST

This kit and all its components are intended to be performed manually or in an open instrument specifically intended for ELISA plate processing.

This kit is a solid phase enzyme immunoassay using 96 coated breakaway microwells and a peroxidase-TMB detection system. The microwells are coated with highly specific antigen.
In the test procedure, serum samples are diluted 1/51 and incubated in the microwells. Human antibodies, if present, bind to the specific antigen. Unbound or excess antibodies are removed by washing and HRP-conjugated rabbit antibodies against human IgA are added to the microwells. The enzyme conjugate binds to the antigen-antibody complexes. After a second washing step to remove excess conjugate, the TMB/substrate solution is added. The enzyme activity, if present, generates a colorimetric (blue) reaction. Diluted acid is added to stop the reaction. Consequently, the colour turns from blue to yellow and may be measured at 450 nm/620 nm using a conventional microplate reader. The absorbance (Optical Density) is directly proportional to the concentration of IgA antibodies bound to the antigen on the microwells surface. The kit is composed of 96 single-use test wells.

3. KIT CONTENTS

Prior to any use of the kit, please check that all the items listed are present or if characteristics of the product are not corresponding to those described hereafter.
If one of the items is missing or damaged or not conforming, please do not use the kit and contact your distributor.

3.1 Components

To be reconstituted: **20x Wash Buffer**	**1 vial, 50 ml - 20 x concentrated (blue)** *Containing: H_2O, TBS, NaCl, Tween, preservatives, dye*
Ready to use: **Sample Diluent**	**1 vial, 50 ml (yellow)** *Containing: H_2O, NaCl, TBS, Tween, BSA, dye, preservatives*
Substrate	**1 vial, 20 ml (colourless)** *Containing: H_2O, TBS, Sodium Acetate, Sodium perborate, stabilizer, EDTA, preservatives*
Negative control	**1 vial, 1 ml (green)** *Containing: human serum (diluted), dye, preservatives*
Calibrators	**6 vials, 1 ml each 0, 25, 50, 100, 200, 400 U/ml.** **(colour increasing with concentration)** *Containing: human serum (diluted), dye, preservatives*
Positive control	**1 vial, 1 ml (blue)** *Containing: human serum (diluted), dye, preservatives*
Conjugate	**1 vial, 20 ml (green)** *Containing: H2O, NaCl, TBS, KCl, HRP conjugate Rabbit anti-human IgA, dye, preservatives*
Stop solution	**1 vial, 20 ml (colourless)** *Containing: sulfuric acid 2.5 %*
Microwell Plate strips	**12 x 8 well strips on a plastic frame with breakaway microwells** *Coated with purified mannan (from S.cerevisiae)*

Abbreviations in alphabetic order:
BSA = Bovine Serum Albumin; EDTA = Ethylenediaminetetraacetic acid; HRP = Horse Radish Peroxidase, KCl = Potassium Chlorure; NaCl = Sodium Chloride; TBS = Tris Buffer Saline; TMB =Tetramethylbenzidine.
For more information on the composition and concentration of the active ingredients used, please refer to the MSDS available on request or on www.d-tek.be.

Symbols used on kit labels

	Attention : consult instructions for use Attenzione : consulti le istruzioni per uso Achtung :Gebrauchsanwendung beachten Attention : consulter le mode d'emploi Atentión : consultar las instrucciones Atenção : consultar instruções para uso Προσοχή : Συμβουλευτειτε τις οδηλιες χρήσης		For ... uses Per ... dosaggi Für ... Anwendungen Pour ... utilisations Para ... usos Para ... utilização για ... χρήσεις
IVD	In vitro diagnostic medical device Dispositivo medico diagnostico in vitro Zur medizinischen diagnostischen Anwendung in vitro Dispositif médical de diagnostic in vitro Dispositivo médico para uso diagnostico in vitro Dispositivo médico para uso diagnostico in vitro Ιατρικό υλικό για διάγνωση In Vitro	REF	Code Codice Artikelnummer Référence Código Código Κωδικός
	To be stored from 2°C to 8°C Conservazione da 2 – 8°C bei 2°C bis 8°C lagern A conserver de 2°C à 8°C Almacenar a 2 - 8°C Armazenar a 2 – 8ºC Αποθηκεύστε στους 2 έως 8°C		Manufactured by Fabbricado da Hergestellt von Fabriqué par Fabricado por Fabricado por Κατασκευάζεται από την
LOT	Batch Number Lotto numero Chargennummer Désignation du lot Denominacion de lote Numéro do lote Κωδικός		Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Verwendbar bis (letzter Tag des Monats) Utiliser avant (dernier jour du mois indiqué) Estable hasta (usar antes de ultimo dia del mes) Data limite para utilização (ultimo dia do mês) Χρήση έως (τελευταια ημέρα του μήνα)
CE	CE Mark Marcatura CE CE-Kennzeichnung Marquage CE Marca CE Marcação CE μονογράφηση CE		To be protected from direct sunlight Proteggere dalla luce Vor Licht schützen Protéger de la lumière Proteja de la luz Proteger da exposição à luz Προστατεύετε τον αντιδραστήριο
WELL	Microwell Pozzetti Kavität Barrette Tira para micropocillo Tira com microcavidades Μικροκοιλοτήτων	CAL ...	Calibrator ... value) Calibratore (... valor) Kalibrator (... Wert) Calibrateurs (... valeur) Calibrador (... valor) Calibrador (... valor) βαθμονομητής (...τιμή)
CONTROL +	Positive control Controllo positivo Positivkontrolle Contrôle positif Controlo positivo Controlo positivo Θετικός μάρτυρας	CONTROL ±	Cut off value Controllo separazione Grenzwertkontrolle Contrôle seuil controlo de corte controlo de redução οριακής τιμής
CONTROL -	Negative control Controllo negativo Negativkontrolle Contrôle négatif Controlo negativo Controlo negativo Αρνητικός μάρτυρας	DIL	Diluent Diluente campione Verdünnungspuffer Diluant Tampón diluyente Tampão de diluição Ρυθμιστικό διάλυμα αραίωσης
WASH ...x	(... x concentrated) wash buffer Tampone di lavaggio (concentrato... x) (... x konzentrierte) Spülpufferlösung tampon de lavage (... x concentré) (... x concentrado) tampones de lavado (... x concentrado) tampão de lavagem (... x συγκέντρωση) Ρυθμιστικό διάλυμα πλύσης	CONJ ...	Conjugate ... Coniugato ... Konjugat ... Conjugué ... Conjugado ... Conjugado ... Συζυγές ...
SUB	Substrate Substrato Substrat Substrat Sustrato Substrato Υπόστρωμα	STOP	STOP solution Soluzione di stop Stopplösung Solution d'arrêt Solución de parada Solução de paragem Διάλυμα διακοπής της αντίδρασης

3.2 Antigen used

S. cerevisiae (Mannan) Saccharomyces cerevisiae phosphopeptido-Mannan purified from S.cerevisiae

4. MATERIAL REQUIRED BUT NOT PROVIDED

- Microtiter plate reader (450 nm reading filter + optional 650 nm reference filter)
- Glass ware, test tubes for the dilutions
- Precision pipettes
- Optional: Microplate washing device (multichannel pipette or automated system)
- Absorbent paper

5. STORAGE

- Store all reagents and microwells at 2-8°C throughout its validity period (see expiration date on the kit). Do not freeze.
- After initial opening of the kit, unused reagents must be stored at 2-8°C protected from (sun)light preferably inside the original kit box. Unused microwell strips have to be placed back into the provided pouches with the absorbent packet, sealed and stored at 2-8°C preferably inside the original kit box. When stored properly, all test kit components are stable until the indicated expiry date.
- Once prepared (refer to 9.2), the washing solution is stable for 1 month at 4°C.

6. SAFETY PRECAUTIONS

1. All reagents are for in vitro diagnostic and professional use only. The test kit should be processed by qualified technical staff only.
2. All human source material used for some reagents of this kit (controls, calibrators) has been tested and found negative for HbsAg, for Hepatitis C and for HIV 1 and 2 antibodies by approved methods. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls, calibrators and patient samples as if capable of transmitting infectious diseases.
3. The reagents in the kit are considered as not dangerous, as the concentrations of potentially dangerous chemicals are below the thresholds specified by European regulations. More information is available on the MSDS of the kit (available upon request or on D-tek website www.d-tek.be).
   Nevertheless, the product contains preservatives which may have (in their given concentration), slightly polluting properties or causing skin sensitization. Therefore, contact with the skin, eyes or mucous membranes should be avoided. As with any chemical containing specific hazards, the product/components of the product should only be handled by qualified personnel and with the necessary precautions.
4. Patient samples should be handled as if they were capable of transmitting infectious diseases; they therefore require suitable protection (gloves, laboratory coat, goggles). In any case, GLP should be applied with all the general or individual safety rules in force.
5. Waste disposal: Patient samples, calibrators and incubated ELISA wells and used reagent vials should be handled as infectious waste. The boxes and other containers do not need to be collected separately, unless stated otherwise in official regulations.

7. RECOMMANDATIONS

1. D-tek and its authorized distributors cannot be held responsible for damages caused indirectly or due to: a change or modification in the indicated procedure, an improper use of the kit and / or the use of an incomplete or damaged kit. The use of this kit is reserved for qualified technical personnel only.
2. D-tek's responsibility is limited in all cases to the replacement of the kit.
3. In the event of a serious incident (injury, deterioration in health, or death) with this IVD device, please report it immediately to the manufacturer (see address below) and to the competent authority in your country.

8. SAMPLE COLLECTION, HANDLING AND STORAGE

The test should preferably be used on recently collected sera samples only! Sera with particles should be centrifuged at low speed. Blood samples should be collected in dry tubes or tubes containing EDTA or heparin. Please avoid using a pool of different sera, as this can lead to inconsistent results (see point 10.4). After separation, the serum samples should be used immediately or aliquoted and stored at 2-8 ° C (for storage for a few days) or frozen at -20°C (for longer storage periods). Repeated freezing/ thawing cycles of the samples must be avoided.

9. ASSAY PROCEDURE

Description of CONTROLS and CALIBRATORS:

No reference material or International standards are available for the anti-s.cerevisiae antibodies.
The **calibrators**, as well as the **Negative and Positive Controls**, consist of a high positive anti-s.cerevisiae sample, prepared in serial dilution. The calibration curve is reflecting the binding kinetic of the antibodies on the immobilized antigen.

The **Cut-Off Control** is calibrated to be the threshold value for the final interpretation of the results (see 10).

9.1 Samples

- Dilute serum samples **1:51** with sample diluent (ready-to-use)
  → **e.g. 500 µl** diluent + **10 µ**l serum. **Mix.**

9.2 Wash buffer

- Dilute the concentrated Wash buffer 1:20 with distilled water
  - ***Manual washing:*** Prepare **10 ml** final volume per **8 wells** or **120 ml** for **96 wells**
    → **e.g. 9.5 ml** water + **0.5 ml** buffer. Mix.
  - ***Automated washing:*** consider excess volumes required for setting up the instrument and dead volume of robot pipette.

9.3 Microwells

- Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store them in the provided plastic bag, sealed tightly

9.4 Pipetting Scheme

- Make sure all reagents are at room temperature before use (18-25°C).

- **Pipette 100 µl** of each patient's **diluted serum** into the designated microwells.
- **Pipette 100 µl calibrators and controls** into the designated wells.
- **Incubate** for **30 minutes** at room temperature (18-25°C).
- **Wash 3 X** with **200 µl washing buffer (diluted 1:20)**.
- **Pipette 100 µl conjugate** into each well.
- **Incubate** for **30 minutes** at room temperature (18-25°C).
- **Wash 3 X** with **200 µl washing buffer (diluted 1:20)**.
- **Pipette 100 µl substrate** into each well.
- **Incubate** for **10 minutes** at room temperature (18-25°C).
- **Pipette 100 µl stop solution** into each well, using the same order as pipetting the substrate.
- **Read absorbance** at **450 nm** (optionally 450/650 nm) within 30 minutes.

NOTE: We recommend to pipette a blank in duplex with each run (sample diluent only, instead of a patient's sample)

Manual washing procedure
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells down-sided vigorously on clean absorbent paper. Pipette 200 µl of diluted wash buffer into each well, wait for 20 seconds, repeat, discard and knock. Repeat the whole procedure twice again.

10. CALCULATION AND INTERPRETATION OF THE RESULTS

10.1 Semi-quantitative interpretation based on calibrators (reflecting the binding kinetic)

Establish the calibration curve by plotting the optical density of each calibrator with respect to the corresponding units' values. The most precise regression model of the calibration curve is the Exponential Association fitting model:

$$y = a(1 - e^{-bx})$$

where y corresponds to the measured O.D. and x corresponds to the arbitrary value in U/ml.

O.D. of each sample (y) can then be calculated in U/ml (x) based on the regression equation.

U/ml	Interpretation
< 20	Negative
20 – 29	Equivocal
>29	Positive

10.2 Semi-quantitative interpretation based on cut off value

A simple semi-quantitative interpretation of the results is possible by using the **25 U/ml** calibrator as a cut off control. Results are expressed in **B**inding **I**ndex, the ratio between the sample and the cut off's O.D.:

B.I. = Sample O.D / Cut-off O.D

A sample is **negative** when **B.I. ≤ 1.0**
A sample is **positive** when **B.I. > 1.0**

10.3 Validation of results

A test run is considered valid if the following Quality Assurance specifications are met.
If not, refer to § 10.5, check the whole procedure and repeat the test. If the problem persists call manufacturer or distributor for assistance.

	Quality Assurance specifications	
	O.D.	**U/ml**
Blank (sample diluent)	< 0.100	-
Negative control	-	≤ 20
25 U/ml calibrator	< 50 % of calibrator 400 U/ml	-
Positive control	> 0.800	200 – 400

10.4 Important recommendations for the interpretation of results

1. D-tek's kits constitute a diagnostic aid. In consequence, no diagnosis can be established solely on the basis of our kits. The results should always be interpreted by taking into account the clinical examination, the patient's history and the results obtained by other methods.
   No single technique can rule out the possibility of false positive or false negative results. With this in mind, an indirect immunofluorescence test should, as far as possible, be carried out prior to the use of a BlueWell kit (immunofluorescence being recognized as a reference method in autoimmunity).

2. The intensity of a result is not necessarily related to the degree of intensity of the disease, but rather to the level of antibodies detected.
3. Low titers of auto-antibodies may occur in healthy patients. For this reason, low positive results (close to the CO, between 20 and 29 U/ml), although valid, should be considered equivocal. In such cases, the retesting of the patient, preferably by using a new sample, is recommended. If the result remains equivocal on retesting, other diagnostic tests and/or clinical information should be used to help determine the autoimmune status of the patient.
4. For various reasons, and under certain conditions, the kit may show a defect in performance (see 10.5 Troubleshooting). In such cases, the results are not valid and cannot be interpreted. It is recommended to repeat the test. If the error persists, please contact your distributor.
5. The intensity of the results may decrease when the device is used at the end of its life. However, the performance of the kit is not affected (detection of positives and negatives) under normal conditions of use and storage.
6. Sequential sampling (at different dates) of an autoimmune patient can sometimes lead to different results from one sample to another. This difference can have several reasons: the patient's treatment, the evolution of the disease, or a seroconversion. In the specific case of seroconversion, the result can be positive for an auto-antibody in an early sampling of the patient, and become positive for another auto-antibody in a later sampling of the same patient.

10.5 Troubleshooting

Problem	**Possible causes + Action**
Discrepancy of results as compared to a reference method	- Use - incorrect pipetting of serum - incorrect volume dispensed - erroneous reading, inappropriate reader filter (use 450 nm or 450/650nm → repeat the test - Use of two different samples of the same patient (see point 10.4.6) or wrong sample handling/storage between tests - Material - Interfering substance in the sample - Sample is a pool of different human sera → repeat the test and confirm by other methods - Method - intrinsic performance of the kit (see 11.2 Analytical sensitivity and specificity) - expired kit - stability problem **Please contact your distributor for any further technical support requests.**
Different results in the same batch or between several batches	- Use - incorrect pipetting of serum - incorrect volume dispensed - - erroneous reading, inappropriate reader filter (use 450 nm or 450/650nm) → repeat the test - Method - intrinsic performance of the kit (see 11.1 Repeatability and Reproducibility)
Contamination between neighbouring wells	- Use - incorrect pipetting of serum / reagents → repeat the test
Poor reaction / O.D too low	- Use - erroneous reading, inappropriate reader filter (use 450 nm or 450/650nm) → repeat the test - damaged reagents → check the integrity of the reagents → contact your supplier if you suspect a problem - wash under-diluted or sample over-diluted → repeat the reagent preparation
Non-specific bindings / high background / O.D. too high	- Material - Interfering substance in the sample → repeat the test and confirm by other methods - Use - wash over-diluted or sample under-diluted → repeat the reagent preparation - excessive incubation time or temperature → repeat the test
Kit not correctly labelled	Manufacturing problem → please contact your distributor
Kit content incorrect	Manufacturing problem → please contact your distributor

NOTE:

The major residual risks of the kit, as given in the risk analysis of the kit at the end of design (after mitigation), are the following:

1) Risk of false results based on a pipetting error (wrong serum)
2) Risk of false results based on an interfering substance contained in the sample

11. PERFORMANCES

11.1 Repeatability and Reproducibility

Reference samples were tested in successive statistically representative series, both in the same test as in different tests and between different batches in order to calculate the intra-assay, inter-assay and inter-lot variations respectively.
In all the cases, the variations in optical density were within the following expected limits:
CV ≤ 10% for intra-assay runs
CV ≤ 15% for inter-assay runs
CV ≤ 20% for inter-lot runs

11.2 Analytical sensitivity

Measurement range: From 0 U/ml (negative) to 400 U/ml (high positive)

Limit of blank (O.D) = 0,099.

As no international standard is available for the auto-antibodies, trueness of measurement is not applicable on this product.

11.3 Analytical specificity

1. The main known interfering substances were tested on the present kit.
   For each concentration of interfering substance tested, the difference between the result of the sample without the interfering substance and the result obtained in the presence of the interfering substance did not exceed 15%.

Interfering substance	Maximum Concentration	Intermediate Concentration	Minimum Concentration	Difference <15%
Bilirubin	100 mg/dL	50 mg/dL	25 mg/dL	Yes
Haemoglobin	200 mg/dL	100 mg/dL	50 mg/dL	Yes
Cholesterol	224.3 mg/dL	112 mg/dL	56 mg/dL	Yes
Rheumatoid factor IgM	~500IU/ml	~300IU/ml	~100IU/ml	Yes

   Note: It is impossible to test all the possible interfering substances described in the literature. Other interferences, amongst others drug-induced interferences, are possible.

2. The high analytical specificity of the test is guaranteed by the quality of the antigen used. This kit detects IgA antibodies against S.cerevisiae. No cross reactions with other autoantibodies have been found.

11.4 Clinical Sensitivity and Specificity

Characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies) were assayed following the test instructions. Sensitivity and Specificity were calculated from the results obtained by external performance evaluations and EQAs control programs. A detailed clinical report is available upon request.

S. cerevisiae IgA

	+	-
	True positive **84**	False positive **5**
	False negative **20**	True negative **281**
Sensitivity	$\frac{84}{104}$ = **81 %**	
Specificity	$\frac{281}{286}$ = **98 %**	

Publication references:

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2. Abu-Freha N, Badarna W, Sigal-Batikoff I, Abu Tailakh M, Etzion O, Elkrinawi J, Segal A, Mushkalo A, Fich A. ASCA and ANCA among Bedouin Arabs with inflammatory bowel disease, the frequency and phenotype correlation. BMC Gastroenterol. 2018 Oct 20;18(1):153. doi: 10.1186/s12876-018-0884-x. PMID: 30342474; PMCID: PMC6195956.
3. Watanabe K, Matsumoto T, Hisamatsu T, Nakase H, Motoya S, Yoshimura N, Ishida T, Kato S, Nakagawa T, Esaki M, Nagahori M, Matsui T, Naito Y, Kanai T, Suzuki Y, Nojima M, Watanabe M, Hibi T; DIAMOND study group. Clinical and Pharmacokinetic Factors Associated With Adalimumab-Induced Mucosal Healing in Patients With Crohn's Disease. Clin Gastroenterol Hepatol. 2018 Apr;16(4):542-549.e1. doi: 10.1016/j.cgh.2017.10.036. Epub 2017 Nov 11. PMID: 29104132.

4. Xiao N, Liu F, Zhou G, Sun M, Ai F, Liu Z. Food-specific IgGs Are Highly Increased in the Sera of Patients with Inflammatory Bowel Disease and Are Clinically Relevant to the Pathogenesis. Intern Med. 2018 Oct 1;57(19):2787-2798. doi: 10.2169/internalmedicine.9377-17. Epub 2018 May 18. PMID: 29780153; PMCID: PMC6207831.
5. Karsten Conrad, Werner Schössler, Falk Hiepe, Marvin J. Fritzler, Book "Autoantibodies in Organ Autoimmune Diseases", Volume 8, second edition – 2017

11.5. Auto-antibody diagnostic value

Anti-S. cerevisiae	Diagnostic marker of *Crohn's Disease*. Anti-ASCA have a sensitivity up to 76%, and a specificity of up to 98% for *Crohn's Disease*. The prevalence in cases of *ulcerative colitis* is low (2-16%). Anti-ASCA are also detectable in newly diagnosed celiac patients, mainly in adults (14-30%). Their level decreases during a gluten-free diet. Anti-ASCA can be found in cases of primary biliary cirrhosis (PBC) (6-24%), *primary sclerosing cholangitis* (44%), *Behçet disease* (42%), *Spondylitis* (19-25%) or in healthy subjects (3-10%).

12. TEST LIMITATIONS

1. The results obtained with this confirmatory test are dependent on the intrinsic performance of the kit and must be considered as an aid to the final diagnosis, taking into account the results obtained by reference techniques and the clinical data of the patient.

2. In case of hyper-lipemic samples, it is recommended to centrifuge it before the pipetting of the 10µl of sample, which must be done into the supernatant.

Version B
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