Arbeitsanleitung / Manual

IDK® Candida albicans IgA ELISA

For the qualitative and semi-quantitative determination of IgA-class antibodies to Candida albicans in serum

Gültig ab / Valid from 2020-10-01

REF **KR3457** Σ 96

REF **KR3457.20** Σ 20 x 96

+2 °C +8 °C

RUO

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1 INTENDED USE

The *IDK*® Candida albicans IgA Enzyme Immunoassay kit provides materials for the qualitative and semi-quantitative determination of IgA-class antibodies to *Candida albicans* in serum.

This assay is intended for research use only (RUO).

2 PRINCIPLE OF THE TEST

The ***IDK*® Candida albicans IgA ELISA** kit is a solid phase enzyme-linked immunosorbent assay (ELISA). Samples are diluted with sample diluent and additionally incubated with IgG-RF-Sorbent, containing hyper-immune anti-human IgG-class antibody to eliminate competitive inhibition from specific IgG and to remove rheumatoid factors. This pretreatment avoids false negative or false positive results.

Microtiter wells as a solid phase are coated with *Candida albicans* antigen. **Pretreated samples** and **ready-for-use controls** are pipetted into these wells. During incubation *Candida albicans*-specific antibodies of positive samples and controls are bound to the immobilised antigens.

After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgA antibodies are dispensed into the wells. During a second incubation this anti IgA conjugate binds specifically to IgA antibodies resulting in the formation of enzyme-linked immune complexes.

After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue colour. The blue colour turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.

The intensity of this colour is directly proportional to the amount of *Candida albicans*-specific IgA antibody in the sample. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

3 KIT OVERVIEW

3.1 Contents of the kit

Cat. No.	Label	Kit components	Quantity for cat. no.	
			KR3457	KR3457.20
KR3457	SAMDIL	Sample diluent*, ready-to-use (coloured yellow, pH 7.2 ± 0.2)	1 x 100 ml	20 x 100 ml

Cat. No.	Label	Kit components	Quantity for cat. no.	
			KR3457	KR3457.20
KR3457	PLATE	Microtiter plate, pre-coated with *Candida albicans* antigen	12 x 8 wells (break apart, incl. 1 strip holder and 1 cover foil)	20 x 12 x 8 wells (break apart, incl. 1 strip holder and 1 cover foil each)
KR3457	IgG-RF-Sorbent	IgG-RF-Sorbent*, ready-to-use (coloured yellow, contains anti-human igG-class antibody)	1 x 6.5 ml	20 x 6.5 ml
KR3457	CTRL POS	Positive control*, ready-to-use (coloured yellow, red cap)	1 x 1 ml	20 x 1 ml
KR3457	CTRL NEG	Negative control*, ready-to-use (coloured yellow, yellow cap)	1 x 2 ml	20 x 2 ml
KR3457	CTRL CUT-OFF	Cut-off control*, ready-to-use (coloured yellow, black cap)	1 x 2 ml	20 x 2 ml
KR3457	CONJ	Enzyme conjugate, ready-to-use* (coloured red, antibody to human IgA conjugated to horse-radish peroxidase)	1 x 20 ml	20 x 20 ml
KR3457	SUB	Substrate, ready-to-use (tetramethylbenzidine)	1 x 14 ml	20 x 14 ml
KR3457	STOP	Stop solution, ready-to-use (contains 0.2 mol/L H_2SO_4)	1 x 14 ml	20 x 14 ml
KR3457	WASHBUF	Wash solution*, 20x, (pH 6.5 ± 0.1)	1 x 30 ml	20 x 30 ml

* contains non-mercury preservative

3.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450/620 nm ±10 nm)
- Calibrated variable precision micropipettes
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Timer
- Absorbent paper

3.2 Storage and stability of the kit

- When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
- Opened kits retain activity for four months if stored as described above.

3.3 Preparation of reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash solution

Dilute wash solution 1+19 (e.g. 10 ml + 190 ml) with fresh and germ-free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2.

Consumption: ~ 5 ml per determination.

Crystals in the solution disappear by warming up to 37 °C in a water bath. **Be sure that the crystals are completely dissolved before use.**

The diluted wash solution is stable for 4 weeks at 2 °C to 8 °C.

4 PREPARATION OF SAMPLES

- Serum can be used in this assay.
- Do not use haemolytic, icteric or lipaemic samples.
- **Please note:** Samples containing sodium azide should not be used in the assay.

4.1 Sample collection

Serum

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Subjects receiving anticoagulant therapy may require increased clotting time.

4.2 Sample storage

- Samples should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.
- Samples held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

4.3 Sample dilution

Prior to assaying each sample is first to be diluted with sample diluent. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with IgG-RF-Sorbent

1. Dilute each subject sample **1+50** with sample diluent;
   e.g. 10 µl of sample + 0.5 ml of sample diluent. Mix well.
2. Mix well IgG-RF-Sorbent before use.
3. Dilute this prediluted sample **1+1** with IgG-RF-Sorbent
   e.g. 60 µl prediluted sample + 60 µl IgG-RF-Sorbent. Mix well.
4. Let stand at room temperature for **at least 15 min, up to a maximum of 2 h** and mix well again.
5. Take 100 µl of these pretreated samples for the ELISA.

Please note: Controls are ready for use and must not be diluted!

5 TEST PROCEDURE

5.1 General remarks

- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**

- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
- During incubation cover microtiter strips with foil to avoid evaporation.

5.2 Assay procedure

Prior to commencing the assay, dilute wash solution, **prepare samples as described in point 4.3**, mix well before pipette and establish carefully the **distribution and identification plan** supplied in the kit for all samples and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.

   Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
1 well	(e.g. B1)	for the negative control,
2 wells	(e.g. C1+D1)	for the cut-off control
1 well	(e.g. E1)	for the positive control.

   It is left to the user to determine controls and samples in duplicate.

2. Dispense

   100 µl of negative control into well B1

   100 µl of cut-off control into wells C1 and D1

   100 µl of positive control into well E1

   100 µl of each pretreated sample with new disposable tips into appropriate wells.

   Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for **60 min at 37 °C**.

4. Briskly shake out the contents of the wells.

   Rinse the wells **5 times** with diluted wash solution (**300 µl per well**). Strike the wells sharply on absorbent paper to remove residual droplets.

   Important note:

   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense **100 µl** enzyme conjugate into each well, **except A1**.

6. Cover wells with foil. Incubate for **30 min at room temperature** (20 °C to 25 °C).

   Do not expose to direct sun light!

7. Briskly shake out the contents of the wells.

   Rinse the wells **5 times** with diluted wash solution (**300 µl per well**). Strike the wells sharply on absorbent paper to remove residual droplets.

8. Add **100 µl** of substrate solution into all wells.

9. Cover wells with foil. Incubate for **exactly 15 min at room temperature** (20 °C to 25 °C) **in the dark**.

10. Stop the enzymatic reaction by adding **100 µl** of stop solution to each well.

    Any blue colour developed during the incubation turns into yellow.

    Note: Highly positive samples can cause dark precipitates of the chromogen!

11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 min** after adding the stop solution.

5.3 Measurement

Adjust the ELISA microplate or microstrip reader **to zero using the substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable **calculate the mean absorbance values** of all duplicates.

6 RESULTS

6.1 Validation of the test run

The test run may be considered valid provided the following criteria are met:

Substrate blank in A1:	Absorbance value **lower than 0.100**
Negative control in B1:	Absorbance value **lower than 0.200**
Cut-off control in C1/D1:	Absorbance value **between 0.350–0.850**
Positive control in E1:	Absorbance value **between 0.650–2.000**

6.2 Calculation of the mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) cut-off control determinations (e.g. in C1/D1).

Example: (0.54 + 0.56) / 2 = 0.55 = CO

6.3 Interpretation

POSITIVE	Subject (mean) absorbance values more than 10 % above CO (Mean OD sample > 1.1 x CO)
GREY ZONE	Subject (mean) absorbance values from 10 % above to 10 % below CO repeat test 2–4 weeks later–with new samples (0.9 x CO ≤ Mean OD sample ≤ 1.1 x CO) Results in the second test again in the grey zone → NEGATIVE
NEGATIVE	Subject (mean) absorbance values more than 10 % below CO (Mean OD sample < 0.9 x CO)

7 QUALITY CONTROL

- It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
- It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
- If the results of the assay do not fit to the established acceptable ranges of control materials subject results should be considered invalid.
- In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
- After checking the above-mentioned items without finding any error contact your *IDK*® directly.

8 ASSAY CHARACTERISTICS

8.1 Assay dynamic range

The range of the assay is between 1.44–60 AU/ml.

$$AU = \frac{\text{Subject (mean) absorbance value x 10}}{CO}$$

8.2 Analytical sensitivity

The analytical sensitivity of the *IDK*® ELISA was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 1.44 AU/ml (OD_{450} = 0.071).

8.3 Diagnostic specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 100 %.

8.4 Diagnostic sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 94.44 %.

8.5 Method comparison

The *IDK*® ELISA was compared to a commercially available CE-marked ELISA.

n = 90		Other commercial ELISA	
		positive	**negative**
IDK® ELISA	positive	17	0
	negative	1	72

8.6 Reproducibility

8.6.1 The intra-assay

The intra-assay (within-run) precision of the *IDK*® ELISA was determined by 20 x measurements of 12 samples covering the measuring range of the ELISA.

Sample	Mean OD	Intra-Assay CV [%]	n
1	0.42	3.63	20
2	0.35	5.65	20
3	0.42	3.59	20
4	0.87	2.97	20
5	0.64	3.47	20
6	0.66	2.61	20
7	1.12	3.59	20
8	1.45	2.88	20
9	1.24	3.42	20
10	2.68	5.66	20
11	2.92	1.43	20
12	2.53	2.29	20

8.6.2 The inter-assay

The inter-assay variation of the *IDK*® ELISA was determined with cut-off control, positive control, and 3 samples with 2 production kits in 10 independent runs with 2 replicates per run.

Sample	Mean OD	Inter-Assay CV [%]	n
1	0.98	6.09	40
2	1.54	4.49	40
3	2.41	3.88	40

9 LIMITATIONS OF USE

Bacterial contamination or repeated freeze-thaw cycles of the samples may affect the absorbance values.

In immunocompromised subject's serological data only have restricted value.

10 GENERAL NOTES AND PRECAUTIONS

- This kit is for research use only (RUO).
- Before starting the assay, read the instructions completely and carefully. **Use the valid version of the package insert provided with the kit.** Be sure that everything is understood.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with stop solution containing 0.2 mol/L H_2SO_4. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. wash contaminated objects before reusing them. If inhaled, take the person to open air.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previ-

ously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.

- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C–26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the subject samples will not be affected.
- Never pipette by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
- Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets.
- Safety Data Sheets for this product are available upon request directly from *IDK*®.

11 REFERENCES/LITERATURE

1. Wingard, J.R., Dick, J.D., Mers, W.G., Sanford, G.R., Saral, A., Burns, W.H.: Pathogenicity of Candida tropicalis and Candida albicans after gastrointestinale inoculation in mice. Infect. Immunol. 29: 808-813, 1980.
2. Stone, H.H., Geheber, C.E., Kolb, L.D., Kitchens, W.R.: Alimentary tract colonization by Candida albicans. J. Surg. Res. 14: 273-276, 1973.

12 SYMBOLS

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet