



NovaLisa™

Toxoplasma gondii IgG Avidity Test

CE 0483

Enzyme immunoassay for the avidity determination of IgG-class antibodies to Toxoplasma gondii in human serum
Enzymimmunoassay zur Bestimmung der Avidität von IgG-Antikörpern gegen Toxoplasma gondii in Humanserum

Only for in-vitro diagnostic use

English:	Page	2 to 4
Deutsch:	Seite	bis
Français:	Page	à
Italiano:	da Pagina	a
Espanol:	Página	a

For further languages please contact our authorized distributors.

Bibliography / Literatur / Bibliographie / Bibliografia / Bibliografía	Page / Seite / Page / Pagina / Página	6
Symbols Key / Symbolschlüssel / Explication des symboles / Legenda / Símbolos	Page / Seite / Page / Pagina / Página	7
Summary of Test Procedure/ Kurzanleitung Testdurchführung/ Résumé de la procédure de test/ Schema della procedura/ Resumen de la técnica	Page / Seite / Page/ / Pagina / Página	8

Product number: TOXGA460 (96 Determinations)

1. INTRODUCTION

The avidity determination is a diagnostic method which is used to differentiate a recent (acute) and a more distant (past) infection with *Toxoplasma gondii* in patient sera. Avidity is the binding force of the antibody (serum specimen) with the corresponding antigen. Low avid IgG antibodies in the early stage of infection can be differentiated from high avid antibodies associated with a past infection. The determination of IgG antibody avidity is an additional analysis to the classic serology in regard to the status of a *Toxoplasma gondii* infection.

2. INTENDED USE

The NovaTec *Toxoplasma gondii* IgG avidity test is intended to differentiate between past and acute infection.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells coated with *Toxoplasma* antigen are incubated with diluted serum specimen (dual pipetting). After washing one well is incubated with avidity reagent and the corresponding well with washing buffer. In this step the low avidity antibodies are removed from the antigens whereas the high avidity ones are still bound to the specific antigens. Anti human IgG labelled with peroxidase is added. The immunocomplex is visualized with TMB to give a blue reaction product. Stop solution is added to stop the reaction and changing the colour of the reaction product into yellow. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

Avidity Reagent* : 1 bottle containing 15 ml of an Urea solution, coloured blue, ready to use, white cap.

Performance control ** : 1 bottle containing 3 ml of a high avidity diluted serum coloured yellow, ready to use, green cap

* contains 0.1 % Bronidox L

** contains 0.02 % Kathon and 0.02% Bronidox

4.2. Additional materials required but not provided

NovaTec CE-labelled *Toxoplasma gondii* IgG ELISA, [REF] TOXG0460

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at +2...+8 °C. After first opening the reagents are stable up to the expiry date.

6. REAGENT PREPARATION

The avidity reagent and performance control are ready to use.

Crystallisation of the avidity reagent may occur at low temperature.

Therefore it is very important to bring the avidity reagent and control to room temperature (+20...+25°C) before starting the test run!

7. ASSAY PROCEDURE

Use procedure and test preparation as mentioned in the NovaTec *Toxoplasma gondii* IgG package insert [REF] TOXG0460

The performance of the avidity test is different as follows:

Dual pipetting of the serum samples and control:

A clean, disposable tip should be used for dispensing the control and serum samples.

1. Wells A1/A2 are used for the substrate blank.
2. Dispense 100 µl performance control in wells B1/B2.
3. Dispense 100µl diluted serum sample (1+100) in wells C1/C2.
Dispense 100µl diluted serum sample (1+100) in wells D1/D2 etc. Cover the wells with foil.
4. **Incubate for 1 hour ± 5 min at 37°C ± 1°C**
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step! (See step 4, package insert *Toxoplasma gondii* IgG, [REF] TOXG0460)
Note: Washing is critical! Insufficient washing results in poor precision.
6. Dispense 100µl of Avidity reagent in wells B1, C1, D1, E1 etc.
Dispense 100µl of diluted (1+19) Washing solution in wells B2, C2, D2, E2 etc.
7. **Incubate for exactly 5 min at room temperature (+20 to +25°C).**
8. Repeat step 5.
9. Dispense 100 µl *Toxoplasma* anti-IgG Conjugate into all wells except in the blank wells (A1/A2). Cover with foil.
10. Following the instructions 6. – 11. mentioned in the package insert of *Toxoplasma gondii* IgG ELISA, [REF] TOXG0460.

8. RESULTS

8.1. Assay validation criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1/A2: Absorbance value **lower than 0.100**.
- **Performance control** in B1/B2: Avidity (%): **exact value and range are indicated on the label**

If these criteria are not met, the test is not valid and must be repeated.

8.2. Calculation of Results

With patient samples having an absorbance value lower than the cut-off determined by the regular NovaTec Toxoplasma gondii IgG ELISA you may not proceed. These samples contain no antibodies to Toxoplasma at all or a concentration of Toxoplasma IgG antibody that is low to evaluate IgG avidity. For each patient sample or control calculate the ratio between the absorbance of the well dispensed with Avidity reagent and the absorbance of the well dispensed with Washing buffer multiplied by 100:

$$\frac{\text{Absorbance (sample or control) Avidity reagent}}{\text{Absorbance (sample or control) Washing buffer (diluted 1 +19)}} \times 100 = \text{Avidity(\%)}$$

Samples or controls with an absorbance greater than the measuring range of the ELISA reader (over f / error) at 450 nm the absorbance of these samples must be read at 405 nm. The calculation of the Avidity (%) is the same as with 450 nm.

8.3. Interpretation of Results

Avidity (%) > 40 Toxoplasmosis antibody with high avidity --> Past infection

Avidity (%) ≤ 40 Toxoplasmosis antibody with low avidity - -> Acute or recent infection

9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1. Precision

Intraassay

Sample	n	Mean avidity(%)	CV (%)
High avidity serum	24	89.0	4.1
High avidity serum	22	77.1	4.0
Low avidity serum	20	24.7	4.6

Interassay

Sample	n	Mean avidity(%)	CV (%)
High avidity serum	6	88.8	2.4
High avidity serum	6	78.8	3.2
Low avidity serum	6	25.8	3.9

9.2. Performance Characteristics

The NovaTec Toxoplasma gondii IgG Avidity Test has been evaluated for use in Toxoplasmosis with acute and past infection sera. A total number of 69 patient samples were tested. These sera were supplied by the Institute of Parasitology, University Bonn.

Total: 69 patient sera

	Acut Infection	Past Infection	Total
Inst. of Parasitology	25	44	69
NovaTec Avidity Test	23	41	64
Discrepancy sera:			
NovaTec Avidity Test	3 f Acut	2 f Past	5
Agreement	92%	93.2%	92.7%

These five discrepant sera were retested in an Avidity ELISA of another manufacturer. Four sera were in accordance to NovaTec Avidity ELISA and 1 serum was different to the NovaTec Test.

9.3. Interferences / Cross reactivity

Regarding the interferences and cross reactivity please refer to the package insert of Toxoplasma gondii IgG [REF] TOXG0460.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

In immunocompromized patients and newborns serological data only have restricted value.

A result of high avidity can not exclude the possibility of a recent infection.

11. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not

liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.

- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- 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 patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The NovaLisa™ Avidity Test is only designed for qualified personnel who are familiar with good laboratory practice.

WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!
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11.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

12. ORDERING INFORMATION

Prod. No.: TOXGA460 Toxoplasma gondii IgG Avidity Test (96 Determinations)

BIBLIOGRAPHY / LITERATUR / BIBLIOGRAPHIE / BIBLIOGRAFIA / BIBLIOGRAFÍA






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Symbols Key/ Symbolschlüssel/ Explication des symboles/ Legenda/ Símbolos	
	Manufactured by / Hergestellt von/ Fabriqué par/ Prodotto da/ Fabricado por
IVD	In Vitro Diagnostic Medical Device/ In Vitro Diagnosticum/ Dispositif médical de diagnostic <i>in vitro</i> / Diganostico <i>in vitro</i> / Producto para diagnóstico In vitro
LOT	Lot Number/ Chargenbezeichnung/ Numéro de lot/ Lotto/ Número de lote
	Expiration Date/ Verfallsdatum/ Date de péremption/ Scadenza/ Fecha de caducidad
	Storage Temperature/ Lagertemperatur/ Température de conservation/ Temperatura di conservazione / Temperatura de almacenamiento
CE	CE Mark/ CE-Zeichen/ Marquage CE / Marchio CE/ Marca CE
[REF]	Catalogue Number/ Katalog Nummer/ Référence du catalogue/ Numero di codice/ Número de Catálogo
	Consult Instructions for Use/ Gebrauchsanweisung beachten/ Consulter la notice d'utilisation/ Consultare le istruzioni/ Consulte las Instrucciones de Uso
	Contains sufficient for "n" tests/ Ausreichend für "n" Tests/ Contenu suffisant pour "n" tests/ Contenido suficiente per "n" saggi/ Contenido suficiente para "n" tests
AVI REAG	Avidity reagent/ Avidity Reagenz
AVI KONT	Performance control/ Funktionskontrolle

SCHEME OF THE ASSAY

Toxoplasma gondii IgG Avidity Test

Assay Preparation

Prepare reagents and samples as described.
 Establish the distribution and identification plan for all specimen and controls on the form supplied in the kit.
 Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Sustrate blank (A1/A2)	Performance control B1	Performance control B2	Sample diluted 1+100 e.g.C1	Sample diluted 1+100 e.g.C2
Performance control- Dual pipetting	-	100µl	100µl	-	-
Sample (1+100) Dual pipetting	-	-		100µl	100µl
Cover wells with foil Incubate for 1 h at 37°C Wash each well three times with 300µl of washing solution					
Avidity reagent	-	100µl		100µl	
Washing solution	-		100µl		100µl
Cover wells with foil Incubate for 5 min at room temperature Wash each well three times with 300µl of washing solution					
Conjugate	-	100µl	100µl	100µl	100µl
Cover wells with foil Incubate for 30 min at room temperature in the dark Wash each well three times with 300µl of washing solution					
TMB	100µl	100µl	100µl	100µl	100µl
Incubate for exactly 15min at room temperature in the dark					
Stop solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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