SERION ELISA *classic* Toxoplasma gondii Avidity Reagent
B110 AVID

SERION ELISA *classic* Avidity Reagents are complementary components which, in combination with the corresponding SERION ELISA *classic*, enables the avidity of target-specific IgG antibodies to be determined. Avidity determination is based on the degradation of low-affinity antibody-antigen complexes; high-affinity complexes are not affected. As a result, low-affinity antibodies are removed during the assay's wash step, and a comparison of parallel assays with and without the avidity reagent allows for the identification of high-affinity antibodies in a sample.

The Avidity Reagents are supplied in lyophilized form. They are reconstituted with distilled water.

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SERION ELISA *classic* Toxoplasma gondii Avidity Reagent

Additive reagent for testing the avidity of human Toxoplasma gondii IgG antibodies.
- For sale in the U.S. for Research Use Only. Not for diagnostic procedures -

Order No.: B110 AVID

1. INTENDED USE

SERION ELISA *classic* Toxoplasma gondii Avidity Reagent is an additional reagent which, in combination with SERION ELISA *classic* Toxoplasma gondii IgG (order no. ESR 129G), enables the avidity of Toxoplasma gondii IgG antibodies to be determined.

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2. SERION ELISA *classic* Toxoplasma gondii Avidity Reagent - TEST PRINCIPLE

Avidity testing of Toxoplasma gondii IgG antibodies is performed using **SERION ELISA *classic* Toxoplasma gondii IgG** (order no. ESR 110G) with the aid of the **SERION ELISA *classic* Toxoplasma gondii Avidity Reagent** (order no. B110 AVID). The avidity reagent causes the disassociation of low affinity antibody-antigen complexes formed on the solid phase during the ELISA test performance while high affinity complexes are not affected. Consequently, low affinity antibodies are removed during the following wash steps, and a comparison of parallel tests with and without the avidity reagent allows for the determination of the avidity of the antibodies present in a sample.

3. KIT COMPONENTS

The avidity reagent is supplied in lyophilized form. The avidity reagent cools substantially as it dissolves, so it is advisable to prepare and/or preheat the reagent (incubator or water-bath at 37°C) approximately 30 minutes before use. The avidity reagent is provided as a single 2.0ml vial.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Common laboratory equipment
- Spectrophotometer for microtiter plates with filter wavelength 405nm, recommended reference wavelength in the range of 620-690 nm (e.g. 650nm).
- Incubator 37°C
- Moist chamber
- Distilled water
- Reference serum for Toxoplasma gondii Avidity Test (order no. BR110AVID)

5. STORAGE AND STABILITY

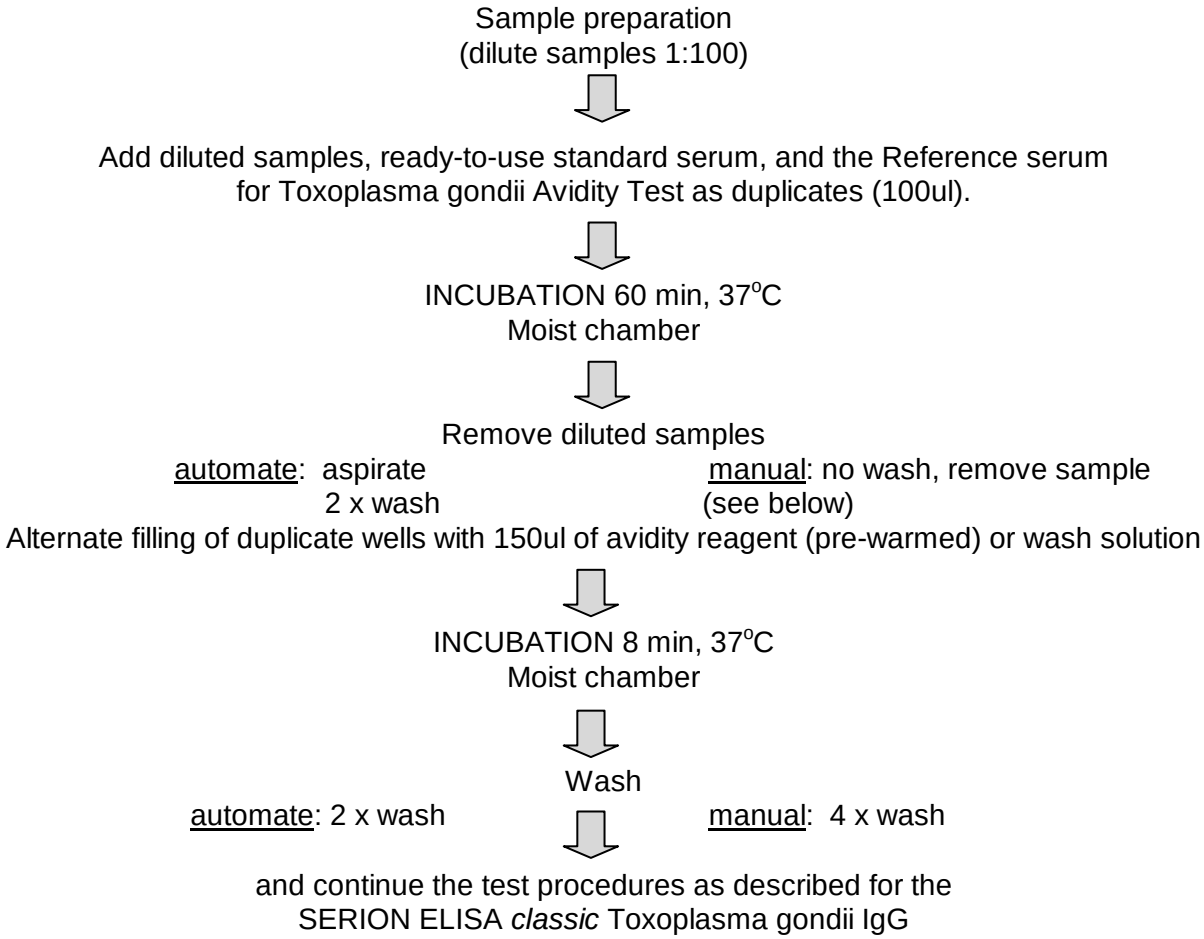
The avidity reagent contains urea which, in aqueous solution, disassociates to ammonia and carbon dioxide. In lyophilized form the avidity reagent is stable until the stated expiration date. Reconstituted liquid reagent should be used as soon as possible (maximum stability 4 weeks at 2-8°C, one year at -20°C). The avidity reagent contains an indicator dye as a pipetting aid.

6. TEST PROCEDURE – AVIDITY TESTING

6.1 General notes

The **SERION ELISA *classic* Toxoplasma gondii Avidity Reagent** is recommended for use only with the **SERION ELISA *classic* Toxoplasma gondii IgG** (order no. ESR 110G). If the reagent is used with ELISAs from other manufacturers, it is not possible to evaluate the results obtained. Please follow the instructions for use supplied with the SERION ELISA *classic* Toxoplasma gondii IgG kit. Reliable results can only be guaranteed if the SERION ELIAS *classic* handling instructions are strictly followed.

6.2 Overview test flow chart



6.3 Test procedure

Diluted samples are dispensed into the SERION EISA *classic* Toxoplasma gondii IgG microtiter plate in a duplicate set-up. After the incubation (60 minutes, 37°C), sample removal is achieved by firmly tapping the inverted microtiter plate on paper towels. The performance of the avidity test differs from the usual SERION ELISA *classic* test procedure only by filling one well with 150ul wash buffer and the other with 150ul avidity reagent in parallel. Following a further incubation of 8 minutes (+/- 1 minute) at 37°C (+/- 1°C), the normal washing steps with wash buffer are followed. Subsequent test steps are performed as described in the instructions for the SERION ELISA *classic* Toxoplasma gondii IgG.

If an automated test processor is used, the washing process has to be modified (see test flow chart). Since shaking out the wells after sample incubation is not possible, the microtiter plate is

washed twice after aspirating the diluted sample. Incubation with avidity reagent or wash buffer then follows and subsequently the remaining two wash steps.

Determination of the IgG antibody titer is not possible in samples with serum activity outside the specified measurement range. Higher serum dilutions (e.g., 1:1,000) should be assessed. The measured antibody activities within the 1:1,000 dilution should be multiplied by the factor 10 in order to calculate the corresponding serum activity.

7. TEST EVALUATION

7.1 Calculation of the SERION avidity indices

Avidity determination with the SERION ELISA *classic* Toxoplasma gondii Avidity Reagent is based on measured signal intensity (OD value) using a formula which takes into account the concentration of specific antibody in samples when calculating the avidity index.

The parameters used in the conversion formula for each individual batch of avidity reagent for use in conjunction with each individual batch of SERION ELISA *classic* Toxoplasma gondii IgG are calculated using results obtained from dilution series of several sera. Additionally, the individual test levels are adjusted to comply with the standard test conditions (target value of standard serum divided by currently measured OD values of standard serum).

$$\text{SERION avidity index} = \frac{\text{OD value (avidity reagent)} \times 100}{\text{OD value (wash buffer)}} X \left[\frac{\text{Target OD value (std)}}{\text{Current OD value (std)}} X \text{ OD value (WB)} \times (-X) + Y \right]$$

A comparison of the precision of the SERION evaluation technique with quantification methods based on antibody concentration showed lower coefficients of variation with the SERION method. For calculation of SERION avidity indeces and determination of high or low avidity, an Excel-based evaluation software SERION *avidity* tool is available on request. When using this tool, the currently determined OD value of the standard serum (not treated with avidity reagent), target value of standard serum, factors A, B, C, D, and X, Y (see the quality control certificate of SERION ELISA *classic* Toxoplasma gondii IgG) have to be entered for every test run.

The SERION evaluation tool delivers further information on samples such as “higher dilution” or “antibody titers too low” if the antibody concentration in the sample is beyond the permitted measurement range. The substrate blank value must be deducted from all measurements results prior to evaluation otherwise the results, particularly for low avidity samples, will be distorted. For users who have photometers which do not automatically deduct blanking values, VIRION SERION can provide an Excel-based program (“Toxoplasma gondii blank”).

7.2 Criteria of validity

- The SERION avidity index of the standard serum must lie within the validity range which is given on the quality control certificate of the SERION ELISA *classic* Toxoplasma gondii IgG. The standard serum is used as an accuracy check for high avidity serum samples.
- AS a control for the low-avidity region, a low avidity serum should also be included, and the results must lie within the expected tolerance range. We recommend the use of the Toxoplasma gondii Avidity test reference serum (order no. BR110AVID) for this purpose. The target values and validity ranges for the SERION Avidity Index are detailed on the accompanying quality control certificate.
- Determination of avidity is not possible for serum samples with antibody concentration below the measurement range.

- For samples with antibody activity above the upper range appropriately higher dilutions than prior testing are necessary.
- Depending on each photometer's individual performance characteristics, higher dilutions should be used if excessively high OD values are obtained, e.g. >2.0.
- The test validity criteria listed in the instructions for use of SERION ELISA *classic* Toxoplasma gondii IgG are applicable. Please note that only the OD values obtained from the well of the Standard serum treated with wash buffer should be used in the evaluation.

8. STATEMENTS OF WARNING

8.1 Statements of warning

The **SERION ELISA *classic* Toxoplasma gondii Avidity Reagent** is an in vitro investigational agent and is only intended for use by qualified personnel who are entirely familiar with the working techniques. Please follow the instructions enclosed with the SERION ELISA classic Toxoplasma gondii IgG and observe the warnings contained therein.

8.2 Disposal

The **SERION ELISA *classic* Toxoplasma gondii Avidity Reagent** is not a hazardous material and can, therefore, be disposed of without any special precautions.