ToRC Avidity Buffer

Buffer Solution for Avidity testing of IgG class of antibodies for ToRC Diagnosis

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

ToRC Avidity Buffer is intended for determining the percentage avidity binding of IgG class of antibodies in Toxoplasma, Rubella and CMV diagnosis using **Bios Microwell ELISA** reagent kits. For in Vitro Diagnostic Use only.

INTRODUCTION

Avidity is a measure of antigen to antibody binding. Avidity Test helps in discriminating primary infection from secondary infection/re-infection when a sample reports positive for both IgG and IgM class of antibodies. Sometimes it is not sufficient to test only for IgM antibodies, as the presence of IgM class of antibodies may be due to the persistence of IgM antibodies from past infection or asymptomatic re-infection without risk for the foetus. For this reason, it is useful to assay the avidity of IgG antibodies. The low avidity of IgG antibodies is therefore an indication of recent or current infection.

PRINCIPLE

ToRC Avidity Buffer acts as a denaturing agent and tends to dissociate the weaker IgG binding with antigen coated onto the microplate. However, if IgG antibodies are persistent against infection for long time a stronger bond is formed with antigen coated onto the microplate and doesn't get dissociated by Avidity buffer. This in turn helps in finding the strength of IgG antibodies in the patient's sample.

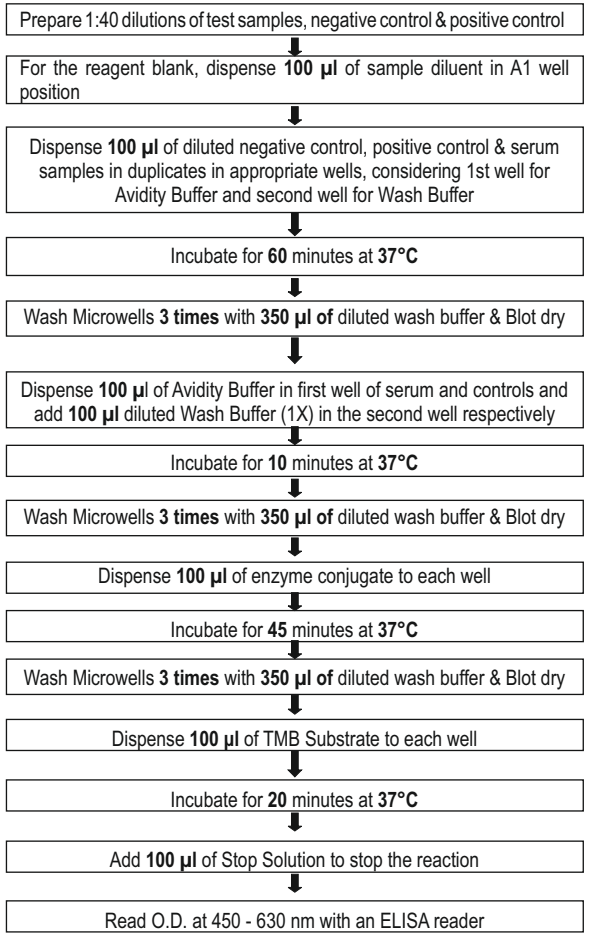
STORAGE AND STABILITY

ToRC Avidity Buffer is stable at 2°C to 8°C up to expiry date printed on the label.

NOTE: ToRC Avidity Buffer is a ready to use reagent and to be used only with Bios Microwell ELISA reagents.

TEST PROCEDURE FOR AVIDITY TESTING

1. Place the desired number of coated strips into the holder. Consider 2 micro wells per sample along with NC and PC.
2. Prepare 1:40 dilutions by adding **5 µl** of the test samples, negative control & positive control to **200 µl** of sample diluent and mix well.
3. For the reagent blank, dispense **100 µl** of sample diluent in A1 well position. Then dispense **100 µl** of diluted negative control, positive control & serum samples in duplicates in appropriate wells, considering 1st well for Avidity Buffer and second well for wash Buffer. Tap the holder to remove air bubbles if any from the liquid. Incubate for **60 minutes at 37°C.**
4. Wash each well three times by filling approximately **350 µl** diluted wash buffer & blot dry.
5. Dispense **100 µl** of Avidity Buffer in first well of each serum sample and controls and add **100 µl** diluted Wash Buffer (1X) in the second well respectively and incubate for **10 minutes at 37°C**.
6. Wash each well three times by filling approximately **350 µl** diluted wash buffer & blot dry.
7. Dispense **100 µl** of enzyme conjugate to each well and incubate for **45 minutes at 37°C**.
8. Wash each well three times by filling approximately **350 µl** diluted wash buffer & blot dry.
9. Dispense **100 µl** of TMB Substrate to each well and incubate for **20 minutes at room temperature**, away from direct light.
10. Add **100 µl** of Stop Solution to stop the reaction.
11. Read O.D. at 450 - 630 nm with an ELISA reader.



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CALCULATION & INTERPRETATION OF THE RESULTS:

The percentage of avidity of the samples is expressed and calculated using a ratio between the O.D of wells containing the Avidity Buffer and those with Wash Buffer, subtracting the value of the blank.

$$\frac{\text{O.D with Avidity Buffer} - \text{O.D of Blank}}{\text{O.D with Wash Buffer} - \text{O.D of Blank}} \times 100 = \% \text{ of Avidity}$$

Ratio > 35% - High Avidity IgG
Ratio < 30% - Low Avidity IgG
Ratio 30-35% - Medium Avidity IgG

IMPORTANT NOTE

1. Inclusion of Blank with sample diluent is mandatory.
2. This assay is a temperature sensitive assay. The best temperature condition for this assay is 37°C.

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SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up	Σ	Contains sufficient for <n> tests
	Do not reuse		

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