

## Avidity Reagent

Test system for determination of the avidity of IgG antibodies

### 1. General aspects

The classical serology of infectious diseases is based on the observation that IgM-antibodies specific to a certain type of pathogen are formed only temporarily, whereas the respective IgG response continues for a long time. For this reason, IgM diagnosis indicates acute infection, but IgG diagnosis without parallel IgM is a sign of past infection. Due to the variability of immune response and to the occurrence of aberrant serological processes, (e.g. persistent, reactivated or absent IgM response) this classical approach may lead to false conclusions in many cases.

Avidity describes the binding strength of specific antibody to antigen. It was found to be low in the first phase after primary infection but then to increase over time. In addition to classic serodiagnosis, measurement of avidity provides information making it possible to distinguish between acute and past infection. During IgG response there is a continuous increase in the avidity of IgG for the respective antigen. This occurs as an immunological principle. For this reason, IgG with a low avidity is usually present in acute infection and IgG with high avidity is present after infection has ended. As a result of this observation, another very significant marker has been introduced into diagnosis in the last few years: the avidity of IgG.

### 2. Test principle

Two test strips are incubated with the diluted serum sample for each test run. During this initial incubation, antibodies in the sample bind to their specific antigens, which are fixed on the test strips. Then one of the two test strips is washed with avidity solution. During this step, the low avid antibodies are removed by diffusion while the high avid antibodies remain bound to their specific antigens. Following the washing step, anti-human antibodies conjugated with horse radish peroxidase are added. The specific antibodies bound to their antigens are made visible by adding a substrate that forms bands on the test strips. The relative position of the colored bands indicates the specificity of the reacting antibodies. Subsequent comparison of the two test strips then provides a basis for measuring the avidity of the antibodies and for establishing the stage of infection.

### 3. Package contents

The reagents in a pack are sufficient for 25 determinations.

Each reagent set contains:

<b>AVID</b>	1	Avidity reagent (solid) for 60 ml ready-to-use solution
<b>INSTRU</b>	1	Instructions for use

### 4. Additional reagents and accessory equipment required

Graduated cylinder, timer

### 5. Precautions

When handling the reagent (solid), equipment designed to protect the respiratory system and eyes must be used and use of clothing designed to protect the skin is also recommended. The ready-to-use solution can be used without respiratory protection. If the reagent comes into contact with eyes or skin it must be rinsed off/out with plenty of water.

## 6. Storage and stability

The stability period of the unopened avidity reagent product (solid) is as indicated on the label. The reagent is stored in the refrigerator (at 2°C – 8°C) or at room temperature. In connection with the particular recomLine kit lot the maximum stability period cannot, however, exceed the kit lot stability period. The avidity reagent (solid) must be protected from moisture.

The ready-to-use solution can be stored in the refrigerator at 2°C – 8°C for 8 weeks. It can be stored at -20°C over a period of 12 months. In this case, the avidity solution should be thawed in warm water (for about 45 minutes).

## 7. Preparation of ready-to-use avidity solution

The weighed-out avidity reagent is dissolved in **40 ml** of the ready-to-use wash and dilution buffer. This dissolving process takes some time and can be accelerated by mild warming. The solution has now a final volume of about 60 ml.

## 8. Test procedure

### 8.1 General

Two parallel strips must always be charged with each sample. To make a comparative evaluation possible, only one of the two strips is then treated with the avidity solution. The reproducibility of the results depends mainly on constant washing of the strips. The washing frequencies as mentioned above should therefore always be complied with.

**The instructions for use of the respective test kit have to be applied also.**

### 8.2 Initial incubation

1. Before use, all reagents should be tempered to room temperature for about 30 minutes (18°C – 25°C). The test procedure is also carried out at room temperature.
2. One well in the incubation tray is required per test charge. **2 ml** of the ready-to-use wash and dilution buffer are pipetted into each incubation well. A test strip is then carefully placed in each of the wells filled with wash buffer using a forceps. The strip number must face upwards.

**Important!**

**Make sure the strips are completely wet and immersed in the liquid.**

3. **20 ul** of an undiluted sample (human serum / plasma) are pipetted into each of two incubation wells for each incubation charge.

The corresponding second charge, which is not to be treated with the avidity solution, must be tested parallel to each sample.

4. The incubation tray is covered with the plastic lid and incubated at room temperature or 1 hour while shaking gently. The incubation temperature should be between 18°C and 25°C.

**Make absolutely sure there is no contamination of adjacent wells, which could lead to false-positive results.**

### 8.3 Washing

1. Following incubation the plastic lids are carefully removed from the incubation trays.

**Important!**

**Make sure the incubation solutions are not entrained into other wells; avoid splashing, especially when opening or closing the lid (danger of cross-contamination).**

2. The reaction solution is carefully aspirated from the individual wells, preferably with a vacuum extraction pipette fitted with a disinfection trap.

**Important!**

**After aspirating the solution from an incubation well, the pipette tips must be changed or rinsed thoroughly with deionised water after each aspiration procedure due to the risk of cross-contamination.**

3. Then place **2 ml** of the ready-to-use wash buffer into each well and incubate for **5 minutes** while shaking gently. The wash buffer is aspirated after the washing procedure.

#### **8.4 Second incubation**

**2 ml** of the ready-to-use avidity solution are added to the first charge and wash buffer is added to the second charge, followed by an incubation for **3 minutes**. In this step, the low-avidity antibodies are "washed off".

#### **8.5 Washing**

The solutions are aspirated from the incubation wells and the strips are washed again, whereby step 3 of section 8.3 is carried out a total of three times.

#### **8.6 Third Incubation**

After the strips are washed, **2 ml** of the appropriately prepared conjugate solution are added to each incubation well and incubated while shaking gently for **45 minutes**, whereby the incubation tray is covered by the plastic lid.

#### **8.7 Washing**

The solutions are aspirated from the incubation wells and the strips are washed again three times (see 8.5).

#### **8.8 Fourth incubation**

1. **1.5 ml** of substrate solution are placed in each well, then incubated for **5 – 15 minutes** at room temperature while shaking gently.
2. After the substrate has been aspirated, the strips are rinsed three times with deionised water.
3. The strips are carefully removed from the water using a forceps and placed on absorbent paper to dry for 2 hours. Then the strips may be adhesively attached to the enclosed evaluation sheet and the results may be entered in the protocol.

### **9. Evaluation**

The evaluation of the avidity strips has to be based on the relevant criteria in the instructions for use for the corresponding parameters.

An antibody is considered to have low avidity if the intensity of the corresponding bands is reduced by approx. 50% in the avidity test. However, the conclusion that any low-avidity antibodies indicate a fresh infection is incorrect. It has been shown that the low-avidity antibodies require different evaluations for each parameter tested. It is therefore absolutely necessary to comply with the instructions for use of each parameter tested for avidity.

Compare the intensities of the corresponding bands on the two test strips incubated with the same sample. Verify that the intensities have changed.

A complete reduction of all intensities is a relatively reliable sign of a fresh infection. Generally, no absolute rules can be set up for avidity evaluation. The interpretation of avidity has to be done always within the context of the overall test results.

### **10. Remarks concerning interpretation**

Deviations from the test procedure as described may falsify the interpretation of the results. It is absolutely necessary to keep incubation times as indicated.

All test results should be considered as supplements to the overall clinical picture. To confirm the diagnosis, the clinical findings and relevant medical history have to be considered as well.