MIKROGEN
DIAGNOSTIK

Avidity Reagent

Test system for determination of the avidity of IgG antibodies
Product no. 11010

1. 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 antibodies. Following the washing step, anti-human antibodies conjugated with horse radish peroxidase are added. The specific antibodies bound to their antigens is 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.

2. Package contents

The reagents in a pack are sufficient for 25 determinations.
Each reagent set contains:

- Avidity reagent (solid25g) for 60 ml ready-to-use solution
- Instructions for use

3. Additional reagents and accessory equipment required:

Metric cylinder with graduations for 50ml ,stop watch

4. 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.

5. 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).

6. Preparation of ready-to-use avidity solution

The weighed-out avidity reagent is dissolved in 40ml 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 60ml.

7. Test procedure

7.1 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.

7.2 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. 2ml 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. 20µl of an undiluted sample 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 plastid lid and incubated at room temperature for 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.

7.3 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 deionized water after each aspiration procedure due to the risk of cross-contamination.

3. Then place 2ml 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.

7.4 Second Incubation
2ml of the ready-to-use avidity solution are added to the first charge and wash buffer are added to the second charge, followed by an incubation for 3 minutes. In this step, the low-avidity antibodies are "washed off." **Important!** It is very important to incubate for exactly three minutes.

7.5 Washing
The solutions are aspirated from the incubation wells and the strips are washed again, whereby step 3 of section 7.3 is carried out a total of three times.

7.6 Third Incubation
After the strips are washed, 2ml 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.

7.7 Washing
The solutions are aspirated from the incubation wells and the strips are washed again three times (see 7.5).

7.8 Fourth Incubation
1. 1.5ml 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 deionized 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.

8. 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.

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 serum. Verify that the intensities have changed.

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.

9. 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.