11. Assay Procedure Continued…

10. Read the optical densities (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

Controls are intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

Plot the OD of each standard against its concentration and draw the best-fit curve through the points. Read the unknowns off this curve. Values below 40 U/ml are considered normal. Values greater than 40 U/ml are considered positive. Samples with values greater than 500 U/ml should be repeated at a higher dilution e.g. 1:200.

14. Limitations of the Procedure

1. Diagnosis cannot be made on the basis of RF ELISA results alone. Results must be interpreted in conjunction with clinical findings.
2. RF can appear transiently during many infections. Patients' positive for RF should be re-tested at an appropriate later date.

15. Performance Characteristics

Clinical Study

Rheumatoid Factor Screen was compared to a latex agglutination assay. The performance of the kit is as follows:

Sample	Latex Titre	GD006 U/ml
1	1:5120	605.7
2	1:2560	143.4
3	1:1280	101.9
4	1:640	75.0
5	1:320	48.8
6	1:160	35.7

16. Reproducibility

Within Assay Precision

CV%: 6.5%

Between Assay Precision

CV%: 6.8%

17. Method Summary

- Dilute sera 1:100 with sample diluent **(Reagent 1)**
- Dispense Standards, the Positive and Negative Controls and the diluted sample into the microplate wells
- Incubate for **30 minutes** at room temperature.
- *Wash the wells three times*
- Dispense 100µl of conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **15 minutes**
- *Wash the wells four times*
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **15 minutes**
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

18. Further Reading

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G·E·N·E·S·I·S Diagnostics

Rheumatoid Factor Screen ELISA Kit

Quantitative assay for IgG, IgA and IgM rheumatoid factor

Product Code: GD006

For *in vitro* Diagnostic Use

CE

1. Materials Included in the Kit

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with rabbit IgG, with holder in a foil bag with desiccant.
- **Reagent 1: Sample Diluent** 10 mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 100ml, (blue), ready to use
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- **Reagent 3: Conjugate** goat anti-human IgAGM (brown) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 25, 50, 100, 200 & 500 U/ml, 1 ml of 10mM Tris-buffered saline containing human RF, ready to use
- **Positive Control:** 1ml of 10mM Tris-buffered saline containing human Rheumatoid Factor; ready to use
- **Negative Control:** 1ml of 10mM Tris-buffered saline containing normal human serum, ready to use.
- **Instructions for use**

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use

The Rheumatoid Factor Screen ELISA kit is a rapid method for the detection of total rheumatoid factor. It is intended as an aid to the diagnosis of rheumatoid diseases. The components of the kits are for *in vitro* diagnostic use only.

4. Explanation of the Test

Rheumatoid factors (RFs) are autoantibodies directed against antigenic sites in the Fc region of human IgG. Elevated RFs are found in 70-90% of patients with rheumatoid arthritis (RA) and also occur frequently in patients with other rheumatic as well as infectious and pulmonary diseases.

Traditional agglutination methods measure principally IgM antibodies. However, patients may have IgA, IgG or IgM RFs, either alone or in combination. Elevated concentrations of IgM RFs are found in approximately 70-80% of patients with confirmed RA and correlate with disease activity and vasculitis. IgM RFs are considered a risk factor in normal subjects. IgA RFs are reportedly associated with bone erosions and symptoms originating from mucosal membranes and secretory organs. A number of studies indicate that the IgA RFs in early disease indicate poor prognosis and justify a more aggressive course of treatment. Elevation in IgA RFs may precede the increase in IgM RF titre. Elevated concentrations of IgG RFs may precede the development of RA and are also considered a risk factor in normal subjects. Raised IgG RFs are virtually confined to the sera of patients with RA and not other arthritides.

5. Principle of the Test

Diluted serum samples are incubated with purified rabbit immunoglobulin IgG immobilised on microtitre wells. After washing away unbound serum components, anti-human IgA-G-M peroxidase conjugate is added to the wells and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, controls and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody activity in the sample.

6. Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8ºC after use.
3. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
4. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
5. Strictly observe the indicated incubation times and temperature.
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 - 8ºC.

9. Specimen Collection and Storage

Serum and plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

Dilute the Wash Buffer **(Reagent 2)** 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

11. Assay Procedure

1. Dilute patient samples 1:100 in Sample Diluent (e.g. 10µl serum plus 1ml diluent).

2. Assemble the number of strips required for the assay.

3. Dispense 100 µl of Sample Diluent as the 0 U/ml Standard. Dispense 100 µl of each Standard, the Negative and Positive Controls and the diluted patient samples into appropriate wells.

4. Incubate for **30 minutes** at room temperature.

5. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

6. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **15 minutes** at room temperature.

7. After 15 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are empty but do not allow to dry out.

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **15 minutes**.

9. Add 100µl of Stop Solution **(Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.