K-*ASSAY*®

RF

For the Quantitative Determination of Human Rheumatoid Factor (RF) in Serum

Cat. No. KAI-031

INTENDED USE

For the quantitative determination of human rheumatoid factor in patient serum based on immunoturbidimetric assay. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

The **K-*ASSAY*®** RF is intended for the quantitative determination of human rheumatoid factor (RF) by immunoturbidimetric assay. The rheumatoid factor, an autoimmune antibody, in the patient's serum interacts with the aggregated human IgG in the reagent forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum rheumatoid factor.

Autoantibodies of the IgG, IgM, or IgA isotype, which are reactive with the crystallizable fraction (Fc) of IgG, are called rheumatoid factors. Rheumatoid factor is found in 50-79% of adults with classical rheumatoid arthritis. Quantification of rheumatoid factor has been shown to be useful in the clinical diagnosis and prognosis of rheumatoid arthritis.[1,2,3,4,5,6,7,8]

Rheumatoid factor has been measured using a variety of methods, including agglutination, latex fixation, nephelometric, and enzyme-linked immunoabsorbent assay.[9,10] The **K-*ASSAY*®** RF uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The **K-*ASSAY*®** RF quantifies the rheumatoid factor in the patient's serum based on immunoturbidimetric assay. Calibrators, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and reagent diluent are automatically pipetted into individual cuvettes.

Following an initial incubation and measurement of sample blank, gamma globulin containing aggregated human IgG is added to the cuvettes. The sample (autoantibodies) solution and gamma globulin reagent (antigen) are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum rheumatoid factor. Following an incubation period lasting approximately 5 minutes, the absorbance of the solution is measured at 340 and 700 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples is interpolated from the calibration curve. Gamma globulin containing aggregated human IgG is used in the kit, which the human rheumatoid factor reacts with.

The **K-*ASSAY*®** RF should be run using the **K-*ASSAY*®** RF Calibrator. Three calibrators are used to prepare a calibration curve for quantifying the levels of rheumatoid factor present in the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent	4 x 20 mL
Tris(hydroxymethyl)aminomethane	50 mM
Sodium Azide	< 0.1%
R2: RF Reagent	2 x 8 mL
Aggregated (denatured) human IgG	0.2%
Tris(hydroxymethyl)aminomethane	100 mM
Sodium Azide	< 0.1%

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

This product contains a chemical known to the State of California to cause cancer. The R-1 reagent contains 2.28% of thiourea (CAS No. 62-56-6).

Reagents contain pooled human serum. Each serum donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for HBsAg and antibody to HIV. However, it is not possible to guarantee that any human source material is free of these infectious agents. Therefore, all products containing human serum should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd ed., 1988, HHS Publication No. (CDC) 88-8395.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for one year from date of manufacture as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard. If the absorbance of the isotonic saline is greater than 0.050 the reagent should not be used. If the calibrator having a rheumatoid factor concentration of around 97 IU/mL does not have an absorbance of 0.050 to 0.200, after subtracting the reagent blank, the reagents should not be used.

Opened reagents can be used for 1 month if stored at 2-8°C.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 340 and 700 nm. Refer to the Instrument Manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to plastic tubes within 2 hours. Freshly drawn serum is preferred. Serum should be stored refrigerated (2-8°C) and used within one week. Samples can also be stored frozen at -20°C and used within 2 months.

Use plastic tubes for storing the sample, do not use glass.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent	4 x 20 mL
Reagent 2 (R-2) RF Reagent	2 x 8 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** RF Calibrator, Cat. No. KAI-032C. (3 calibrators, approximate values: 50, 150, 300 U/mL)

Purified water

Two-reagent clinical chemistry analyzer capable of accurate absorbance readings at 340 and 700 nm, accurately dispensing the required volumes, and maintaining 37°C.

Assay Procedure

An example of automated application (Hitachi 717):

Sample		15 µL
↓		
• ←	R1 (Buffer Reagent)	250 µL
↓	37°C, 5 min.	
• ←	R2 (RF Reagent)	50 µL
↓	37°C, 5 min.	
2-point endpoint, 340/700 nm		

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(RF)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(15) ( )
R1 VOLUME	(250) ( ) (NO)
R2 VOLUME	(50) ( ) (NO)
WAVELENGTH	(700) (340)
CALIB. METHOD	(NONLINEAR) (4) (4)
STD.(1) Conc.-POS.	(0.0) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(0) - (0)
STD.(6) Conc.-POS.	(0) - (0)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.00)

* 2-4 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

For samples with RF concentrations less than 320 IU/mL, a multi-point calibration curve using the ***K-ASSAY®*** RF Calibrator (Cat. No. KAI-032C) should be used. It is recommended that the user determine calibration frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be done every day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the stated recovery range.

LIMITATIONS OF PROCEDURE

The measuring range for Rheumatoid Factor is between 5 IU/mL and 320 IU/mL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1:2 with isotonic saline or filtered to decrease nonspecific light scattering. If rheumatoid factor concentration in a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensation for dilution.

PERFORMANCE

Precision

The precision for the ***K-ASSAY®*** RF was determined using packaged reagents, pooled human serum, and a Hitachi model 717 chemistry analyzer.

Precision Assay:
(Within Run)

Sample I	Sample II
N = 20	N = 20
Mean = 3.3	Mean = 110.15
SD = 0.86	SD = 1.5
CV = 26.197%	CV = 1.359%

Precision Assay:
(Between Runs)

Sample I	Sample II	Sample III
N = 10	N = 10	N = 10
Mean = 7.9	Mean = 33	Mean = 106.3
SD = 2.13	SD = 1.33	SD = 2.06
CV = 26.98%	CV = 4.04%	CV = 1.94%

Accuracy / Correlation

y = 0.876x - 20.268
r = 0.984
n = 43
x = company A's ITA
y = ***K-ASSAY®*** RF

Assay Range

5 - 320 IU/mL

INTERFERENCE

Bilirubin F and C	No interference up to 20 mg/dL
Hemoglobin	No interference up to 500 mg/dL
Lipemia	No interference up to 3%

EXPECTED VALUE

The expected value as reported is below 10 U/mL or below 11 IU/mL. Each laboratory should establish its own expected values using this kit.

REFERENCES

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8. Peter, J.B. "Use and interpretation of tests in clinical immunology," Specialty Laboratories, Santa Monica, CA , 8th edition, 1991.
9. Killingsworth, L.M. and Savory, J. "Nephelometric studies on the precipitin reactions," J. Clin. Chem. 19:403-407, 1973.
10. Sternberg, J.C. " A Rate Nephelometer for Measuring Specific Proteins by Immunoprecipitin Reaction," Clin. Chem., 23:1456-64, 1977.

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

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