K-*ASSAY*®

RF (Ver.2)

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

Cat. No. KAI-230

INTENDED USE

The **K-*ASSAY*®** RF (Ver.2) assay is for the quantitative determination of human IgG rheumatoid factor antibodies in patient serum or plasma (citric acid, EDTA, or lithium heparin) based on immunoturbidimetric assay. The presence of IgG RF antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis (RA). FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

The **K-*ASSAY*®** RF (Ver.2) assay is intended for the quantitative determination of human IgG rheumatoid factor (RF) antibodies by immunoturbidimetric assay. The rheumatoid factor (an autoimmune antibody in the patient's serum or plasma) 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 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 (Ver.2) uses a latex-enhanced immunoturbidimetric assay format.

PRINCIPLE OF TEST

The **K-*ASSAY*®** RF (Ver.2) assay quantifies the rheumatoid factor in the patient's serum or plasma based on latex-enhanced 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 rheumatoid factor. Following an incubation period lasting approximately 5 minutes, the absorbance change of the solution is measured.

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 controls and patient samples are interpolated from the calibration curve.

The **K-*ASSAY*®** RF (Ver.2) assay should be run using the **K-*ASSAY*®** RF Calibrator (Ver.2). These 6 calibrators are used to prepare a calibration curve for quantifying the levels of rheumatoid factor present in the patient's serum or plasma sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 1 x 20 mL
Phosphate Buffer

R2: Latex Suspension 1 x 20 mL
Suspension of latex particles sensitized with human γ globulin (0.7 mg/mL)
Phosphate Buffer

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 contain human source material. Each 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 or other infectious agents.

Therefore, all products containing human source material should be handled in accordance with good laboratory practices and appropriate control. See the National Institute of Health Manual, "Biosafety in Microbiology and Biomedical Laboratories," 2nd ed., 1988.

Reagents in this kit contain < 0.1% w/v 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 Center for Disease Control, Atlanta, GA.

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 until the expiration date indicated 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.

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 absorbances. 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 CLSI document M29-A3. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum or plasma (EDTA or lithium heparin) test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (4-8°C) for 8 days or at -20°C for up to 3 months.[11] Avoid repeat freeze/thawing of specimens.

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

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent 1 x 20 mL
Reagent 2 (R-2) Latex Suspension 1 x 20 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** RF Calibrator (Ver.2),
Cat. No. KAI-231C.

Purified water

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Assay Procedure

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

An example of automated application:

Sample 3.0 µL
↓
• ←R1 (Buffer Reagent) 100 µL
↓ 37 °C, 5 min.
• ←R2 (Latex Suspension) 100 µL
↓ 37 °C, 5 min.
2-point endpoint, 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Abbott Architect c8000
ASSAY NAME	(RFv2)
ASSAY TYPE	(PHOTOMETRIC)
ASSAY AVAILABILITY	(ENABLED)
REACTION MODE	(END UP)
WAVELENGTH	(700) / (NONE)
LAST READ REQUIRED	(33)
ABSORBANCE RANGE	( ) ( )
SAMPLE BLANK TYPE	(SELF)
FLEX READ TIME	(32) - (33)
COLOR CORRECTION READ TIME	( ) - ( )
BLANK READ TIME	(19) - (20)
REAGENT	(RF)
DILUENT	(NONE)
DILUTION DISP. MODE	(TYPE 1)
REAGENT VOLUME	R1:(100) R2:(100)
WATER VOLUME	R1:( ) R2:( )
DISPENSE MODE	(TYPE 1) (TYPE 1)
DILUTION NAME	(STANDARD)
SAMPLE	(3.0)
DILUTION FACTOR	( )
DEFAULT DILUTION	(SELECTED)
REACTION CHECK	(RATE RATIO)
MAX. ABS. VARIATION	(0.01)
CALIBRATION METHOD	(SPLINE)
REPLICATES	(3)
RESULT CONC. UNITS	(IU / ML)

RF Calibrators (Ver.2) includes 6 levels to be used as calibrators. Input the concentrations for each calibrator.

Parameters for other automated analyzers are available.

CALIBRATION

For samples with RF concentrations less than 600 IU/mL, a multi-point calibration curve using the **K-*ASSAY*** ® RF Calibrator (Ver.2), Cat. No. KAI-231C, should be used. In our testing on an Abbott Architect c8000, the calibration curve was stable for 43 days. It is recommended that the user confirm calibration frequency as this may depend on the instrument and type/number of other assays being performed.

TRACEABILITY

The values of the **K-*ASSAY*** ® RF Calibrator (Ver.2) are traceable to the NIBSC Rheumatoid Arthritis Serum, 64/002. Based upon this standardization, results are reported in International Units (IU/mL).

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 6.65 to 600 IU/mL.

Grossly lipemic samples and samples known to have very high triglyceride concentrations should be diluted with isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by the dilution factor.

If the 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 (Ver.2) assay was determined using packaged reagents, clinical serum samples, and an Abbott Architect c8000 chemistry analyzer according to the CLSI EP5-A3 guideline. Representative data are below.

Single Site Precision		Sample			
		1	2	3	4
Mean (IU/mL)		13.72	22.22	101.38	519.46
N		80	80	80	80
Repeatability	S.D.	0.57	0.41	0.56	2.58
	C.V. %	4.1	1.8	0.6	0.5
Within-Laboratory	S.D.	0.57	0.50	0.83	3.60
	C.V. %	4.2	2.2	0.8	0.7

Multi-Site Precision		Sample			
		1	2	3	4
Mean (IU/mL)		14.13	21.24	101.69	532.83
N		75	75	75	75
Repeatability	S.D.	0.64	0.54	0.75	4.44
	C.V. %	4.5	2.5	0.7	0.8
Reproducibility	S.D.	1.02	1.03	1.61	7.86
	C.V. %	7.2	4.9	1.6	1.5

Accuracy / Correlation

Testing was performed on an Abbott Architect c8000 analyzer according to the CLSI EP9-A3 guideline. A comparison of the **K-*ASSAY*** ® RF (Ver.2) assay and company A's RF assay was performed with the following results:

y = 1.001x + 1.331
r = 0.989
n = 178
x = company A's RF
y = **K-*ASSAY*** ® RF (Ver.2)

x min	= 5.1	y min	= 6.77
max	= 558.8	max	= 550.92
mean	= 124.5	mean	= 128.94

Detection Limit

LoB Limit of Blank	LoD Limit of Detection	LoQ Limit of Quantitation
2.21 IU/mL	4.29 IU/mL	6.65 IU/mL

Linearity

The regression equation for the linear range (6.65 - 600 IU/mL) was: y = 0.992x + 2.41 with an r value of 0.9986.

Hook Effect / Prozone

No hook effect was observed up to 1,700 IU/mL.

Matrix comparison:

Fifty-nine patient samples were collected, with each sample being processed to make 4 samples of Serum, Citric Acid Plasma, Li-Heparin Plasma, and EDTA Plasma.

The regression equations are as follows (Serum = y):

Serum vs Citric Acid Plasma:
$y = 0.9854x - 0.4601$, $R^2 = 0.9929$

Serum vs Li-Heparin Plasma:
$y = 0.9897x + 1.4617$, $R^2 = 0.9985$

Serum vs EDTA Plasma:
$y = 0.9971x - 0.2719$, $R^2 = 0.9985$

Assay Range

6.65 - 600 IU/mL

INTERFERENCE

Testing was performed on an Abbott Architect c8000 analyzer according to the CLSI EP07-3rd edition guideline with the following results.

Ascorbic Acid	No interference up to 50 mg/dL
Bilirubin, Conjugated	No interference up to 20 mg/dL
Bilirubin, Unconjugated	No interference up to 20 mg/dL
Chyle	No interference up to 1,500 FTU
Hemoglobin	No interference up to 500 mg/dL
Total Cholesterol	No interference up to 400 mg/dL
Total Triglycerides	No interference up to 1,000 mg/dL

EXPECTED VALUE

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

REFERENCES

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LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostics 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

EC REP

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