AssayLite™

Human Albumin and Rat Albumin Multiplex EFCIA Kit

(Red and Blue Fluorescent Probes)

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

This product is manufactured under patented technology by Assaypro LLC

US Patent No. 9,945,847

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

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Assay Summary

Step 1. Add 50 µl of Standard/Sample and 50 µl of Fluorescent Probe per well.
Incubate 2 hours at 37°C.

Step 2. Wash, then add 50 µl of Stabilizing Solution per well.

Step 3. Human Albumin: Read at EX 485/20 nm, EM 575/15 nm
Rat Albumin: Read at EX 590/10 nm, EM 660/10 nm

Symbol Key

Consult instructions for use.

Assay Mechanism

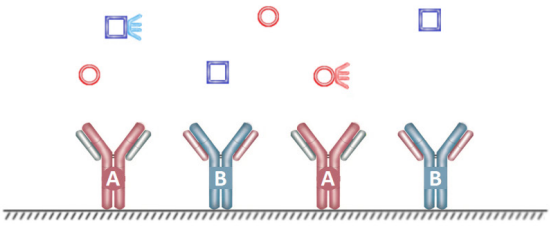


Figure 1. Two different types of antibodies are coated uniformly on the microplate. Standard or Sample and the Fluorescent Probe are added to each well.

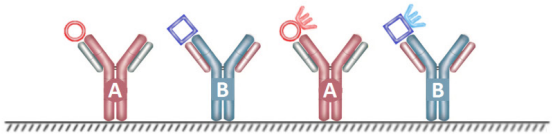
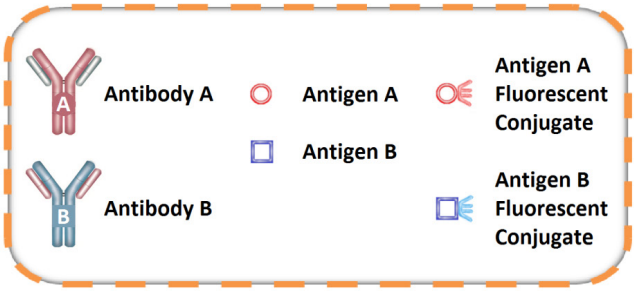


Figure 2. Fluorescent Probe competes with the non-labeled protein for binding to the antibody. The blank (Diluent only, 0 µg/ml) shows the highest fluorescent signal. Signal decreases when the non-labeled protein concentration increases.



Assay Template

	A	B	C	D	E	F	G	H
1								
2								
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Human Albumin and Rat Albumin Multiplex EFCIA Kit

Catalog No. FA320111M1

Sample insert for reference use only

Introduction

Albumin, the main protein in plasma, is a globular unglycosylated serum protein with a molecular weight of 65 kDa that is synthesized by the liver. The preproalbumin contains 609 amino acids and is processed to 585 amino acids in the mature protein (1). It comprises three homologous domains that assemble to form a heart-shaped molecule. Each domain is a product of two subdomains that possess common structural motifs (2). Albumin regulates blood oncotic pressure or colloidal osmotic pressure and transports hydrophobic molecules, such as lipids, hormones, and toxins. It is also an important circulating antioxidant and possesses enzymatic properties (3). Serum albumin level has been linked in clinical practice to several diseases. Low albumin levels can suggest liver disease, kidney disease, inflammation, shock, and malnutrition (4-6). On the other hand, high albumin levels usually reflect dehydration (7).

Principle of the Assay

The AssayLite™ Human Albumin and Rat Albumin Multiplex EFCIA (Endpoint Fluorescent Competitive Immunoassay) Kit employs a **quantitative competitive fluorescent probe technique** that measures albumin in human and rat **plasma and serum samples** in approximately 2 hours. A polyclonal antibody specific for human albumin and a polyclonal antibody specific for rat albumin have been pre-coated onto a 96-well opaque polystyrene microplate with removable strips. Human and rat albumin in standards and samples are competed with a human albumin and rat albumin fluorescent probe. All unbound material is washed away before the endpoint fluorescence is measured.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (diluent buffer, wash buffer, standard, and fluorescent probes) as instructed, prior to running the assay.

- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.
- Avoid direct light exposure to the assay.
- Store fluorescent probes in a dark place. Do not freeze.

Reagents

- **Human/Rat Albumin Microplate:** A 96-well opaque polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human albumin and a polyclonal antibody against rat albumin.
- **Sealing Films:** Each kit contains 3 precut, pressure sensitive aluminum sealing films that can be cut to fit the format of the individual assay.
- **Human/Rat Albumin Standard:** Human/Rat albumin in a buffered protein base (Human: 750 µg, Rat: 300 µg, lyophilized).
- **Red Fluorescent Human Albumin Probe (1x):** 3 vials, lyophilized.
- **Blue Fluorescent Rat Albumin Probe (1x):** 3 vials, lyophilized.
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml, 1 bottle).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 1 bottle).
- **Stabilizing Solution (1x):** A solution to stabilize the fluorescent component (8 ml, 1 bottle).

Storage Conditions

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Microplate, Diluent Concentrate (10x), Stabilizing Solution, and Wash Buffer at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.
- Store Fluorescent Probes in a dark place at 2-8°C. Do not freeze.

Other Supplies Required

- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37°C)
- Fluorescent Microplate Reader

Fluorescent Microplate Reader (BioTek® Synergy H1F, filter-based reader; available for order from Assaypro)

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. A 20000-fold sample dilution is suggested into MIX Diluent *(suggested dilution factor only; user should determine optimal dilution factor depending on application needs).* The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. A 20000-fold sample dilution is suggested into MIX Diluent *(suggested dilution factor only; user should determine optimal dilution factor depending on application needs).* The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

  Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample: 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.
- **Human/Rat Albumin Standard:** Reconstitute the Human/Rat Albumin Standard (Human: 750 µg, Rat: 300 µg) with 1.5 ml of MIX Diluent to generate a standard stock solution (Human: 500 µg/ml, Rat: 200 µg/ml). Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (Human: 500 µg/ml, Rat: 200 µg/ml) 4-fold with MIX Diluent to produce a standard curve that measures both human and rat albumin proteins simultaneously. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[Human] (µg/ml)	[Rat] (µg/ml)
P1	1 part Standard	500	200
P2	1 part P1 + 3 parts MIX Diluent	125	50
P3	1 part P2 + 3 parts MIX Diluent	31.25	12.5
P4	1 part P3 + 3 parts MIX Diluent	7.813	3.125
P5	1 part P4 + 3 parts MIX Diluent	1.953	0.781
P6	MIX Diluent	0.0	0.0

- **Red Fluorescent Human Albumin Probe (1x):** Reconstitute the fluorescent probe with 1 ml of MIX Diluent to produce a stock solution. Allow the probe to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at 2-8°C and used within 5 days. Do not freeze.
- **Blue Fluorescent Rat Albumin Probe (1x):** Reconstitute the fluorescent probe with 1 ml of MIX Diluent to produce a stock solution. Allow the probe to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at 2-8°C and used within 5 days. Do not freeze.

    - After Red and Blue Fluorescent Probes are reconstituted and ready, combine equal parts of both probes to the desired volume to generate the **Human/Rat Albumin Fluorescent Probe Mixture**.

Example:
Combine the following reagents according to the number of wells in the assay (n) plus one well.

2450 µl Red Fluorescent Human Albumin Probe
2450 µl Blue Fluorescent Rat Albumin Probe
Assuming the needed volume is less than or equal to 4900 µl
(96 wells + 1 well)

- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.

Assay Procedure

- Prepare all reagents, standard solution, and samples as instructed. Bring all reagents to room temperature before use. The assay is incubated at 37°C.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human/Rat Albumin Standard or sample to each well, and immediately add 50 µl of Human/Rat Albumin Fluorescent Probe Mixture to each well (on top of the standard or sample). Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing film and incubate for 2 hours at 37°C. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Immediately add 50 µl of Stabilizing Solution to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
- **Human Albumin:** Read the endpoint fluorescence on a microplate reader at an excitation wavelength of 485/20 nm and emission wavelength of 575/15 nm.
- **Rat Albumin:** Read the endpoint fluorescence on a microplate reader at an excitation wavelength of 590/10 nm and emission wavelength of 660/10 nm.
- **Human Albumin:** For the Synergy H1F, a gain of 75 is suggested; however, the user should determine the optional gain/amplification.

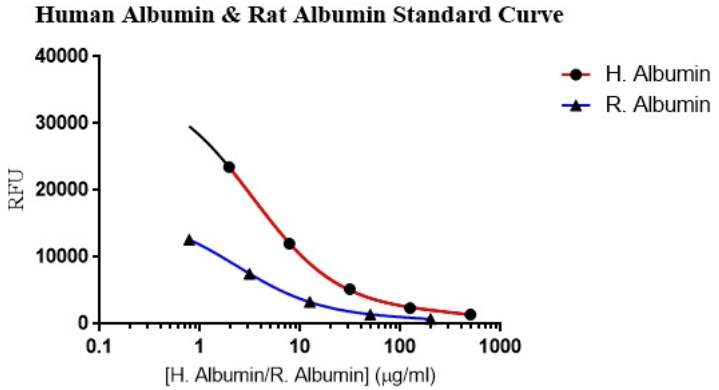
- **Rat Albumin:** For the Synergy H1F, a gain of 90 is suggested; however, the user should determine the optional gain/amplification.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean emitted fluorescence on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Note: Standard curve reading with Biotek® Synergy H1F

Performance Characteristics

Reader	Standard Point	µg/ml	Human Albumin Average Red RFU
BioTek – Synergy H1F	P1	500	1400
	P2	125	2379
	P3	31.25	5215
	P4	7.813	12021
	P5	1.953	23479
	P6	0.0	38943

Reader	Standard Point	µg/ml	Rat Albumin Average Blue RFU
BioTek – Synergy H1F	P1	200	740
	P2	50	1421
	P3	12.5	3310
	P4	3.125	7511
	P5	0.781	12629
	P6	0.0	16406

- The minimum detectable dose of human albumin as calculated by 2SD from the mean of a zero standard was established to be 1.2 µg/ml.
- The minimum detectable dose of rat albumin as calculated by 2SD from the mean of a zero standard was established to be 0.6 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 7.0% and 12.3% respectively.

Reference Value

- Normal human albumin plasma levels range from 30 to 50 mg/ml.
- Normal rat albumin plasma levels range from 20 to 50 mg/ml.
- Human and rat plasma samples were tested (n=30).

Sample	n	Human Albumin Average Value (mg/ml)	Rat Albumin Average Value (mg/ml)
Pooled Normal Plasma	30	37.6	38.3

Recovery

Name	Human Albumin	Rat Albumin
Standard Added Value	7.8 – 125 µg/ml	3.1 – 50 µg/ml
Recovery %	96 – 111%	92 – 108%
Average Recovery %	98%	101%

Linearity

Average Percentage of Expected Value (%)		
Sample Dilution for Plasma	Human Albumin	Rat Albumin
10000x	90%	92%
20000x	99%	98%
40000x	105%	106%

Cross-Reactivity

Species	Cross-Reactivity (%)
Bovine	None
Canine	None
Mouse	<1%
Monkey	None
Rat	100%
Rabbit	None
Swine	None
Human	100%

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the Standard and Fluorescent Probe after reconstitution. • Thoroughly mix dilutions.

	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
	Prolonged exposure of assay or Fluorescent Probe to light	• Overexposure can affect the stability of the Fluorescent Probe, store in a dark location. • Cover and cap all reagents when not in use. • Cover assay with aluminum sealing film immediately after loading.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporated	• Verify that the aluminum sealing film is firmly in place before placing the assay in the incubator.
	Used filters with an overlapping bandpass	• As an example, do not use a filter combination of 620/20 EX and 660/40 EM, use a 660/20 filter instead.
Deficient Standard Curve Fit	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the Standard and Fluorescent Probe after reconstitution. • Thoroughly mix dilutions.

References

(1) Minghetti PP *et al.* (1986) *J Biol Chem.* 261(15):6747-6757.
(2) He XM, Carter DC. (1992) *Nature.* 358(6383):209-215.
(3) Minchiotti L *et al.* (2008) *Human Mutation.* 29(8):1007-1016.
(4) Schindler C *et al.* (1999) *J Hepatol.* 31(6):1132.
(5) Hemmelder MH *et al.* (1997) *Nephrol Dial Transplant.* 12 Suppl 2:57-62.
(6) Wettstein R *et al.* (2004) *Shock*. 22(4):351-357.
(7) Strand TA. (2004) *Am J Clin Nutr*. 79(3):451-456.

Fluorescent Microplate Reader

- BioTek® Synergy H1F, Filter-Based Reader
- Gen5 Software included
- Includes three filters designed specifically for AssayLite® Multiplex Assays. Additional filters are available for purchase
- Available for order from Assaypro for $19,950