Rat Galectin-3 (LGALS3) ELISA

Catalog Number ERLGALS3 (96 tests), ERLGALS3X10 (10 x 96 tests)

Rev. 8

Product description

The Rat Galectin-3 (LGALS3) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of Rat Galectin-3 in cell culture supernatants, plasma and serum.

Contents and storage

Store the kit reagents at -20°C. Expiry of the kit and reagents is stated on the labels. The kit components' expiry is guaranteed only if they are stored properly and not contaminated during repeated use. Do not mix components from different kit lots.

Components	Cat. No. ERLGALS3 (96 tests)	Cat. No. ERLGALS3X10 (10 x 96 tests)
Rat Galectin-3 Antibody Coated wells, 96-well plate (12 strips of 8 wells)	1 plate	10 plates
Rat Galectin-3 Biotin Conjugate (80X)	2 vials	20 vials
Rat Galectin-3 Standard, recombinant Rat Galectin-3	2 vials	20 vials
Wash Buffer Concentrate (20X)	25 mL	12 x 25 mL
Assay Diluent B (5X)	15 mL	10 x 15 mL
Streptavidin-HRP (200X)	0.2 mL	10 x 0.2 mL
TMB Substrate	12 mL	10 x 12 mL
Stop Solution	8 mL	10 x 8 mL
Adhesive Plate Covers	2	20

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm (620 nm as optional reference wavelength)
- Beakers, flasks, and cylinders for preparation of reagents
- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Statistical calculator with program to perform regression analysis
- Microplate shaker
- Plastic wrap

Prepare 1X Wash Buffer

1. Allow Wash Buffer Concentrate (20X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 20 mL of the Wash Buffer Concentrate into 380 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within one month.

Prepare 1X Assay Diluent

- Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

Prepare 1X Biotin Conjugate

1. Briefly spin down the concentrated biotin conjugate before use.
2. Add 100 µL of 1X Assay Diluent B into the vial to prepare a biotin conjugate concentrate.
3. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days).
4. The biotin conjugate concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 2 of ELISA procedure.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

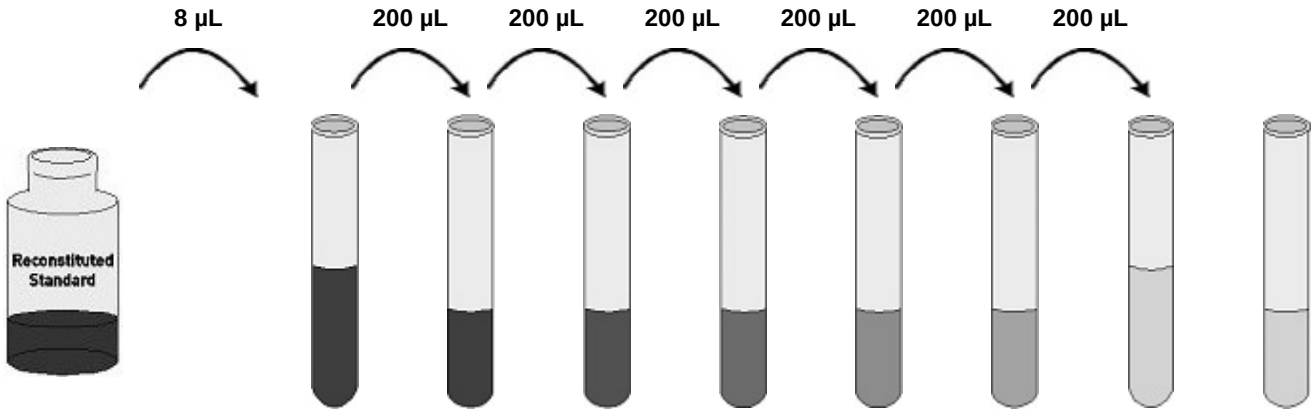
Pre-dilute samples

- 1X Assay Diluent B should be used for dilution of serum, plasma, and cell culture supernatant samples.
- Dilute **serum and plasma samples** 2 - 20 fold.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Briefly spin down a vial of lyophilized standard.
2. Add 400 µL 1X Assay Diluent B (should be diluted 5-fold with deionized or distilled water before use) into the lyophilized standard vial to prepare a 50 ng/mL standard solution. Dissolve the powder thoroughly by gentle mixing. Add 8µL Galectin-3 standard from the vial of tem C, into a tube with 492µL 1X Assay Diluent to prepare an 800 pg/mL standard solution. Pipette 300 µL 1X Assay Diluent B into each tube. Use the 800 pg/mL standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the zero standard (0 pg/mL).



	Diluent volume	**492 µL**	**300 µL**	**300 µL**	**300 µL**	**300 µL**	**300 µL**	**300 µL**	**300 µL**
		Std 1	**Std 2**	**Std 3**	**Std 4**	**Std 5**	**Std 6**	**Std 7**	**Blank**
50 ng/mL		**800.0 pg/mL**	**320.000 pg/mL**	**128.000 pg/mL**	**51.200 pg/mL**	**20.480 pg/mL**	**8.190 pg/mL**	**3.280 pg/mL**	**0 pg/mL**

Prepare 1X Streptavidin-HRP solution

Note: Prepare the Streptavidin-HRP within 15 minutes of usage.

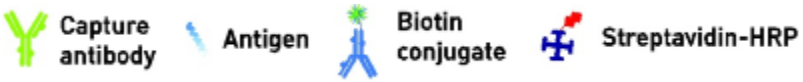
1. Briefly spin the Streptavidin-HRP and pipette up and down to mix gently before use, as precipitates may form during storage and inadequate mixing can raise effective Streptavidin-HRP concentration and produce higher signal.
2. Dilute Streptavidin-HRP 200-fold with 1X Assay Diluent B. For example: Briefly spin the vial and pipette up and down to mix gently. Add 50µL of HRP-Streptavidin concentrate into a tube with 10mL 1X Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin solution (Use the diluted Strept-HRP solution same day; Do not store diluted solution for future use). Mix well.
3. Do not store diluted solution for future use.

Perform ELISA (Total assay time: 4 hours and 45 minutes)

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use.

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



1. Bind antigen

a. For the standard curve, add 100 µL of standards to the appropriate wells (see Dilute standards). For samples, add 100 µL of diluted samples (see Dilute samples) to the wells.
b. Cover the plate with plastic wrap and incubate for 2.5 hours at room temperature or cover it with the adhesive plate covers and incubate overnight at 4°C with gentle shaking at 40 rpm.
c. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration. Do not scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively, microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

2. Add biotin conjugate

a. Add 100 µL of prepared 1X biotin conjugate (see Prepare biotin conjugate) to each well.
b. Cover the plate with plastic wrap and incubate for 1 hour at room temperature with gentle shaking at 40 rpm.
c. Repeat the wash as in step 1c.

3. Add Streptavidin-HRP

a. Add 100 µL of prepared Streptavidin-HRP solution (see Prepare Streptavidin-HRP solution) to each well.
b. Cover the plate with plastic wrap and incubate for 45 minutes at room temperature with gentle shaking at 40 rpm.
c. Repeat the wash as in step 1c.

4. Add TMB substrate

a. Add 100 µL of TMB Substrate to each well. The substrate will begin to turn blue.
b. Cover the plate with plastic wrap and incubate for 30 minutes at room temperature **in the dark** with gentle shaking at 40 rpm.

5. Add stop solution

a. Add 50 µL of Stop Solution to each well. Tap the side of the plate gently to mix. The solution in the well changes from blue to yellow. Read OD immediately after adding Stop Solution because OD values can change during longer waiting times.

Read the plate and generate the standard curve

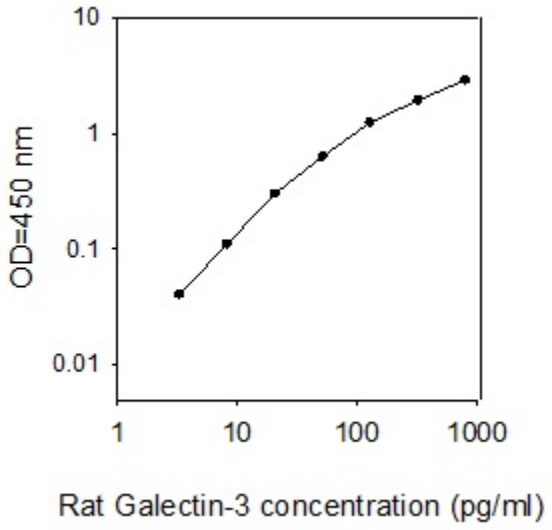
1. Read the absorbance at 450 nm. Read the plate within 30 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and control from the standard curve. Multiple value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer, or shorten incubation time, and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

These standard curves are for demonstration only. A standard curve must be run with each assay.



Intra-assay precision

To determine intra-assay precision, two standard curves and 3 samples for each standard curve are run. The standard curve concentration points as well as the samples are tested in duplicates on a single plate. Two different concentration values are obtained for each sample, using the two separate standard curves. The two concentration values for each sample is compared to each other using the CV% calculation.
Intra-Assay CV: <10%

Inter-assay precision

To evaluate inter-assay precision, the second standard curve is tested on a separate plate along with the second set of samples.
Inter-Assay CV: <12%

Recovery

Sample Type	Average % Recovery	Range (%)
Cell Culture Supernatants	79	72-85
Plasma	88	67-134
Serum	99	94-113

Specificity
This ELISA antibody pair detects rat and mouse Galectin-3.

Linearity of dilution
The cell culture supernatants, plasma and serum samples were spiked with recombinant Rat Galectin-3, serially diluted in Assay Diluent B and evaluated. Observed values were compared to expected values to calculate percent recovery and demonstrate the dilution linearity of the assay.

Sample Type	Average % Expected		Range (%)	
	1:2 Dilution	1:4 Dilution	1:2 Dilution	1:4 Dilution
Cell Culture Supernatants	128	134	122-134	126-143
Plasma	127	114	112-144	107-123
Serum	114	112	106-122	83-103

Sensitivity
The minimum detectable dose of Rat Galectin-3 is 3 pg/mL. This was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

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REF Catalog number
LOT Batch code
Temperature limitation
Use by
Manufacturer
Consult instructions for use
Caution, consult accompanying documents

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