Instructions for Use

For the quantitative measurement of Human Galectin-3 concentrations in cell culture supernatant, serum and plasma (EDTA)

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

## INTRODUCTION
1. BACKGROUND 2
2. ASSAY SUMMARY 4

## GENERAL INFORMATION
3. PRECAUTIONS 5
4. STORAGE AND STABILITY 5
5. MATERIALS Supplied 5
6. MATERIALS REQUIRED, NOT SUPPLIED 6
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

## ASSAY PREPARATION
9. REAGENT PREPARATION 8
10. STANDARD PREPARATIONS 12
11. SAMPLE COLLECTION AND STORAGE 14
12. PLATE PREPARATION 15

## ASSAY PROCEDURE
13. ASSAY PROCEDURE 16

## DATA ANALYSIS
14. CALCULATIONS 19
15. TYPICAL DATA 20
16. TYPICAL SAMPLE VALUES 21
17. ASSAY SPECIFICITY 22

## RESOURCES
18. TROUBLESHOOTING 23
19. NOTES 24
INTRODUCTION

1. BACKGROUND

Abcam’s Galectin-3 Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate quantitative measurement of Human Galectin-3 concentrations in cell culture supernatant, serum and plasma (EDTA).

Galectin-3 specific antibodies have been precoated onto 96-well plates. Standards and test samples are added to the wells along with a biotinylated Galectin-3 detection antibody and the microplate is then incubated at room temperature. Following washing with wash buffer a Streptavidin-HRP conjugate is added to each well, incubated at room temperature and unbound conjugates are then washed away using wash buffer. TMB is then added and catalyzed by HRP to produce a blue color product that changes to yellow after addition of an acidic stop solution. The density of yellow coloration is directly proportional to the amount of Galectin-3 captured in plate.

Galectin-3 is a 26kDa β-galactoside-binding protein belonging to the Galectin family, which consists of more than ten members. Galectin-3 is composed of a carboxyl-terminal carbohydrate recognition domain (CRD) and amino-terminal tandem repeats. Galectin-3 normally distributes in epithelia of many organs and various inflammatory cells, including macrophages as well as dendritic cells and Kupfer cells. The expression of this lectin is up-regulated during inflammation, cell proliferation and cell differentiation and through transactivation by viral proteins. Its expression is also affected by neoplastic transformation: up regulation is found in certain types of lymphomas, and thyroid carcinoma, while it is down regulated in other types of malignancies such as colon, breast, ovarian and uterine carcinomas. The expression of Galectin-3 has a strong correlation with the grade and malignant potential of primary brain tumors. Increased Galectin-3 levels have also been noted in Human atherosclerotic lesions. These findings suggest that Galectin-3 expression is affected during these physiological and pathological responses. Galectin-3 has been shown
to function through both intracellular and extracellular actions. It is a component of heterogeneous nuclear ribonuclear protein (hnRNP), a factor in pre-mRNA splicing and has been found to control cell cycle and prevent T-cell apoptosis through interaction with the Bcl-2 family members. On the other hand, this protein, which is secreted from monocytes/macrophages and epithelial cells has been demonstrated to function as an extracellular molecule in activating various types of cells such as monocytes/macrophages, mast cells, neutrophils and lymphocytes. Galectin-3 has been shown to mediate cell-cell and cell-extracellular matrix interactions and acts as a novel chemoattractant for monocytes and macrophages.
2. **ASSAY SUMMARY**

**Primary Capture Antibody**

Prepare all reagents, samples and standards as instructed.

**Sample**

Add standard or sample to each well used.

**Detection Antibody**

Add prepared detection antibody to each well. Incubate at room temperature.

**Streptavidin-HRP**

Wash and add prepared Streptavidin-HRP conjugate. Incubate at room temperature.

**Substrate**

Wash and add TMB Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Upon receipt, store kit immediately at 2-8°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9 Reagent Preparation.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microplate coated with monoclonal antibody to Galectin-3 (12 x 8 wells)</td>
<td>96 wells</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Biotin Conjugated anti- Galectin-3 polyclonal antibody</td>
<td>100 µL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Streptavidin-HRP</td>
<td>150 µL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Fibronectin Standard lyophilized (50 ng/mL upon reconstitution)</td>
<td>2 Vials</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>12 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>20X Assay Buffer Concentrate</td>
<td>5 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>50 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>TMB Substrate Solution</td>
<td>15 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Stop Solution (1M Phosphoric acid)</td>
<td>15 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Blue-Dye</td>
<td>400 µL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Green-Dye</td>
<td>400 µL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Red-Dye</td>
<td>400 µL</td>
<td>2-8 ºC</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- As exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radio immunotherapy has significantly increased the number of patients with Human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine polyclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or polyclonal antibodies of irrelevant specificity) are added to the sample.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 1X Wash Buffer

Prepare 1X Wash Buffer by diluting the 20X Wash Buffer Concentrate with distilled or deionized water. To make 500 mL 1X Wash Buffer, combine 50 mL 20X Wash Buffer Concentrate with 450 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

*Note: The 1X Wash Buffer should be stored at 2-8 °C and is stable for 30 days.*

9.2 1X Assay Buffer

Prepare 1X Assay Buffer by diluting the 20X Assay Buffer Concentrate with distilled or deionized water. To make 50 mL 1X Assay Buffer, combine 5 mL 20X Assay Buffer Concentrate with 45 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

*Note: The 1X Assay Buffer should be stored at 2-8 °C and is stable for 30 days.*
9.1 **1X Biotin Conjugated Antibody**

To prepare the Biotin Conjugated Antibody, dilute the anti-Human Galectin-3 polyclonal antibody 100-fold with 1X Assay Buffer. Use the following table as a guide to prepare as much 1X Biotin Conjugated Antibody as needed by adding the required volume (µL) of the Biotin Conjugated Antibody to the required volume (mL) of 1X Assay Buffer. Mix gently and thoroughly.

<table>
<thead>
<tr>
<th>Number of strips</th>
<th>Volume of Biotin-Conjugated Galectin-3 antibody (µL)</th>
<th>1X Assay Buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>30</td>
<td>2.97</td>
</tr>
<tr>
<td>7 - 12</td>
<td>60</td>
<td>5.94</td>
</tr>
</tbody>
</table>

*Note: The 1X Biotin-Conjugated Antibody should be used within 30 minutes after dilution.*

9.2 **1X Streptavidin-HRP Conjugate**

To prepare the Streptavidin-HRP Conjugate, dilute the anti-Streptavidin-HRP Conjugate 400-fold with 1X Assay Buffer. Use the following table as a guide to prepare as much 1X Streptavidin-HRP Conjugate as needed by adding the required volume (µL) of the Streptavidin-HRP Conjugate to the required volume (mL) of 1X Assay Buffer. Mix gently and thoroughly.

<table>
<thead>
<tr>
<th>Number of strips</th>
<th>Volume of Streptavidin-HRP (µL)</th>
<th>1X Assay Buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>15</td>
<td>5.985</td>
</tr>
<tr>
<td>7 - 12</td>
<td>30</td>
<td>11.97</td>
</tr>
</tbody>
</table>

*Note: The 1X Streptavidin-HRP should be used within 30 minutes after dilution.*
9.3 **Optional**: Addition of Color-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help kit users to avoid any pipetting errors when performing the ELISA, this kit offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the kit user with the performance of the test, but can also be omitted, just following the protocol booklet.

Alternatively, the dye solutions from the stocks provided *(Blue-Dye, Green-Dye, Red-Dye)* can be added to the reagents according to the following guidelines:

### 9.3.1 Blue Dye addition to Sample and Standard Diluent

Before diluting standards and samples in the appropriate diluent, the Blue-Dye can be added to the diluents at a dilution of 1:250 (see table below)

After addition of Blue-Dye, proceed according to the protocol booklet.

<table>
<thead>
<tr>
<th>Volume of 1X Diluent (mL)</th>
<th>Volume of Blue Dye (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td>50</td>
<td>200</td>
</tr>
</tbody>
</table>
9.3.2 **Green Dye addition to 1X Assay Buffer (for dilution of Biotin-Conjugated Antibody)**

Before diluting the Biotin-Conjugate anti-Human galectin-3 polyclonal antibody in 1X Assay Buffer, the Green-Dye can be added to at a dilution of 1:100 to an aliquoted volume of the 1X Assay Buffer (see table below). Proceed after addition of Green-Dye according to the protocol booklet.

<table>
<thead>
<tr>
<th>Volume of 1X Assay Buffer (mL)</th>
<th>Volume of Green Dye (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
</tr>
</tbody>
</table>

9.3.3 **Red Dye addition to 1X Assay Buffer (for dilution of Streptavidin-HRP)**

Before dilution of the concentrated Streptavidin-HRP, add the Red-Dye at a dilution of 1:250 (see table below) to the 1X Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of Red-Dye according to the protocol booklet: Preparation of Streptavidin-HRP.

<table>
<thead>
<tr>
<th>Volume of 1X Assay Buffer (mL)</th>
<th>Volume of Red Dye (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
</tr>
</tbody>
</table>
10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Prepare a 50 ng/mL Stock Standard by reconstituting one vial of the galectin-3 standard with the volume of distilled water stated on the label. Hold at room temperature for 10-30 minutes. The 50 ng/mL Stock Standard cannot be stored for later use.

10.2 Label eight tubes with numbers 1 - 8.

10.3 Add 225 µL Sample Diluent to all tubes.

10.4 Prepare a 25 ng/mL Standard 1 by adding 225 µL of the 50 ng/mL stock to tube 1. Mix thoroughly and gently.

10.5 Prepare Standard 2 by transferring 225 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare Standard 3 by transferring 225 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes number 4 through to 7.

10.8 Standard 8 contains no protein and is the Blank control.
## ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (ng/mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>225</td>
<td>225</td>
<td>50.00</td>
<td>25.00</td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>225</td>
<td>225</td>
<td>25.00</td>
<td>12.50</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>225</td>
<td>225</td>
<td>12.50</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>225</td>
<td>225</td>
<td>6.25</td>
<td>3.13</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>225</td>
<td>225</td>
<td>3.13</td>
<td>1.56</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>225</td>
<td>225</td>
<td>1.56</td>
<td>0.78</td>
</tr>
<tr>
<td>7</td>
<td>Standard 6</td>
<td>225</td>
<td>225</td>
<td>0.78</td>
<td>0.39</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>-</td>
<td>225</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Stock solution
2. Standard 1
3. Standard 2
4. Standard 3
5. Standard 4
6. Standard 5
7. Standard 6
8. None

![Diagram of assay preparation](image-url)
11. SAMPLE COLLECTION AND STORAGE

- Cell culture supernatant, serum and plasma (EDTA) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

- Possible “Hook Effects” may be observed due to high sample concentrations. It is recommended to run several dilutions of your sample to ensure an accurate reading.

- Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

- Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Human Galectin-3. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.

- Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

- Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the Human galectin-3 levels determined. There was no significant loss of Human Galectin-3 immunoreactivity detected by freezing and thawing.

- Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the Human galectin-3 level determined after 24 h. There was no significant loss of Human galectin-3 immunoreactivity detected during storage under above conditions.
12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use.
- Unused well strips should be returned to the plate packet and stored at 2-8°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections. Determine the number of microplate strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards.

13.2. Wash the microplate twice with approximately 400 µL 1X Wash Buffer per well with thorough aspiration of microplate contents between washes. Allow the 1X Wash Buffer to remain in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microplate.

13.3. After the last wash step, empty wells and tap microplate on absorbent pad or paper towel to remove excess 1X Wash Buffer. Use the microplate strips immediately after washing. Alternatively the microplate strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

13.4. Add 100 µL of prepared standards (including the standard blank control) to the appropriate wells.

13.5. Add 50 µL of Sample Diluent to the sample wells.

13.6. Add 50 µL of samples to appropriate wells.

13.7. Add 50 µL of 1X Biotin Conjugated Antibody to all wells.

13.8. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 2 hours (microplate can be incubated on a shaker set at 400 rpm).

13.9. Remove adhesive film and empty wells. Wash microplate strips 3 times according to step 13.2. Proceed immediately to step 13.10.
13.10. Add 100 µL of 1X Streptavidin-HRP to all wells, including the blank wells.

13.11. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour (microplate can be incubated on a shaker set at 400 rpm).

13.12. Remove adhesive film and empty wells. Wash microplate strips 3 times according to step 13.2. Proceed immediately to the next step.

13.13. Pipette 100 µL of TMB Substrate Solution to all wells.

13.14. Incubate the microplate strips at room temperature (18 to 25°C) for 10 minutes. Avoid direct exposure to intense light.

*Note:* The color development on the plate should be monitored and the substrate reaction stopped (see step 13.15) before the signal in the positive wells becomes saturated. Determination of the ideal time period for color development should be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 - 0.95.

13.15. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well.

*Note:* It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microplate strips are stored at 2 - 8°C in the dark.

13.16. Read absorbance of each microplate on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader.
ASSAY PROCEDURE

according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

*Note:* In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.
14. CALCULATIONS

Average the duplicate readings for each standard, sample and control blank. Subtract the no protein control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

If samples have been diluted 1:2, as stated in step 13.5, the concentration obtained from the standard curve must be multiplied by the dilution factor (x 2) to obtain an accurate value, in addition to any initial sample dilution factor.

Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human Galectin-3 levels (Hook Effect). Such samples require further external predilution according to expected human Galectin-3 values with Sample Diluent in order to precisely quantitate the actual human Galectin-3 level.
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>O.D. 450 nm Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>0.39</td>
<td>0.054</td>
</tr>
<tr>
<td>0.78</td>
<td>0.082</td>
</tr>
<tr>
<td>1.56</td>
<td>0.169</td>
</tr>
<tr>
<td>3.13</td>
<td>0.262</td>
</tr>
<tr>
<td>6.25</td>
<td>0.736</td>
</tr>
<tr>
<td>12.50</td>
<td>1.585</td>
</tr>
<tr>
<td>25.00</td>
<td>3.176</td>
</tr>
</tbody>
</table>

Figure 1. Example of Human galectin-3 protein standard curve.
16. **TYPICAL SAMPLE VALUES**

**SERUM/PLASMA –**

Panels of 16 serum samples and 10 plasma samples (EDTA) were tested for Human Galectin-3. The detected Human Galectin-3 levels in serum samples ranged between 0.0 and 2.28 ng/ml with a mean level of 0.54 ng/ml. The detected Human Galectin-3 levels in plasma samples (EDTA) ranged between 4.67 and 10.30 ng/ml with a mean level of 7.07 ng/ml.

**SENSITIVITY -**

The limit of detection of Human Galectin-3 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.12 ng/ml (mean of 6 independent assays).

**RECOVERY –**

The spike recovery was evaluated by spiking 4 levels of Human Galectin-3 into pooled normal serum samples. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. The recovery ranged from 73% to 88% with an overall mean recovery of 77%.

**LINEARITY OF DILUTION –**

3 serum samples with different levels of Human Galectin-3 were analyzed at serial 2 fold dilutions with 4 replicates each.

The recovery ranged from 94% to 128% with an overall recovery of 115%.
DATA ANALYSIS

PRECISION –
Intra- and Inter-assay reproducibility was determined by measuring samples containing different concentrations of Human galectin-3.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>%CV</td>
<td>6.4</td>
<td>11.4</td>
</tr>
</tbody>
</table>

17. ASSAY SPECIFICITY
The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a Human Galectin-3 positive serum. There was no cross reactivity detected.
## 18. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Samples give higher value than the highest standard</td>
<td>Starting sample concentration is too high.</td>
<td>Dilute the specimens and repeat the assay</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the kit</td>
<td>Store the all components as directed.</td>
</tr>
</tbody>
</table>
19. NOTES
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp