

AlphaLISA COL1A1 (Human) Detection Kit

Product number: AL371 HV/C/F

Lot number: 2643680

Manufacturing date: October 17, 2019

Research Use Only. Not for use in diagnostic procedures.

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Product Information

- Application:** This kit is designed for the quantitative determination of Human Collagen, Type I, Alpha 1 (COL1A1) in human serum, plasma, cell lysate, and cell culture supernatants using a homogeneous AlphaLISA assay (no wash steps). The assay shows no cross-reactivity with mature human collagen Type II, Type III, fibronectin, and hyaluronic acid. Cross reactivity with other species has not been tested.
- Sensitivity:** Lower Detection Limit (LDL): 6 pg/mL
Lower Limit of Quantification (LLOQ): 20 pg/mL
EC₅₀: 59 ng/mL
- Dynamic range:** 10 – 300 000 pg/mL (Figure 1).

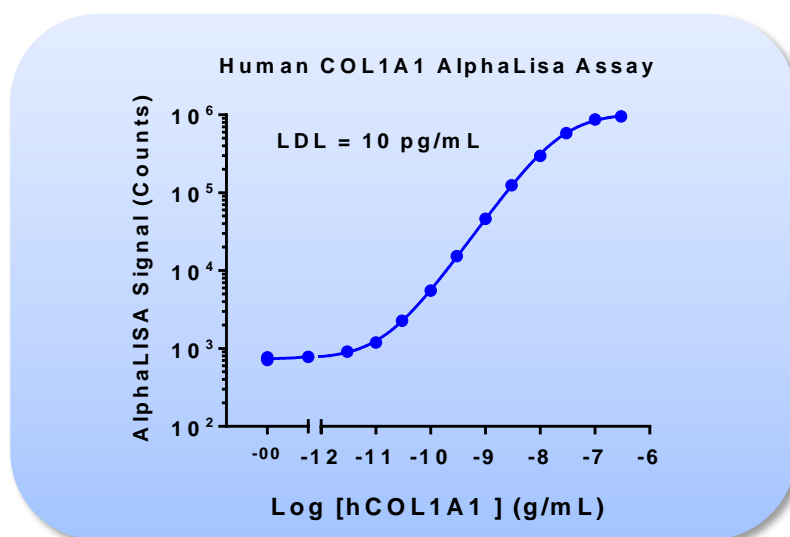


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2103 with Alpha option.

- Storage:** Store kit in the dark at +4°C. Store analyte at 4°C for up to 4 weeks or store long term at -20°C.
- Stability:** This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC₅₀ and LDL were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on LDL measurement.

| | |
|--------------------|--------------|
| EC ₅₀ : | 19.500 ng/mL |
| LDL: | 2.883 pg/mL |
| LLOQ: | 9.580 pg/mL |
| Min Counts: | 398 |
| Max Counts: | 338,931 |

Analyte of Interest

Collagen, type I, alpha 1 (COL1A1) is known as alpha-1 type I collagen that is produced by COL1A1 gene in the connective tissues. Upon synthesis as the pro-alpha1(I) chains, two pro-alpha1(I) chains and one a pro-alpha2(I) chain combine to make up rope-like procollagen molecules that are ultimately converted into Type I collagen by enzymes outside the cells. Type I collagen is well known to maintain and support connective tissues such as cartilage, bone, tendon, and skin. Changes of COL1A1 levels in cells, connective tissues, and in biological fluids are associated with diseases like Ehlers-Danlos syndrome, osteogenesis imperfecta, and osteoporosis. The present kit is designed to detect COL1A1 in human serum, plasma, cell culture supernatants, cell lysates, and tissue homogenates.

Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In an AlphaLISA assay, a Biotinylated Anti-Analyte Antibody binds to the Streptavidin-coated Alpha Donor beads, while another Anti-Analyte Antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

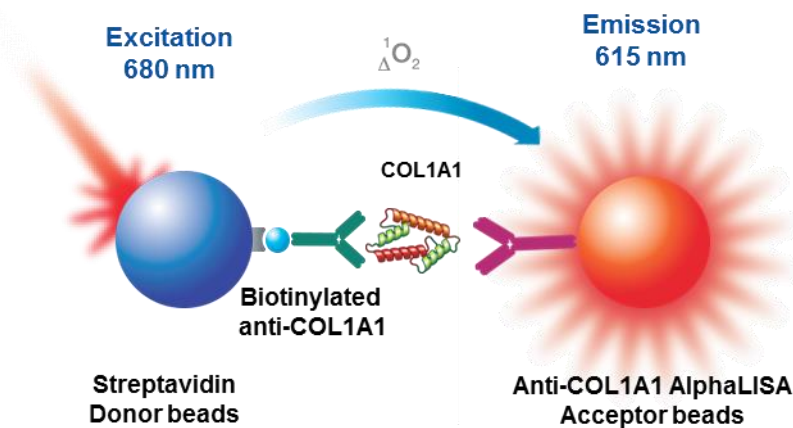


Figure 2. AlphaLISA Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous. The analyte included in this kit is from a human source.
- Some analytes are present in saliva. Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

Kit Content: Reagents and Materials

| Kit components | AL371HV (100 assay points ^{***}) | AL371C (500 assay points ^{***}) | AL371F (5000 assay points ^{***}) |
|---|---|--|--|
| AlphaLISA Anti-hCOL1A1 Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2 | 20 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap) | 50 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap) | 500 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap) |
| Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4 | 80 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap) | 200 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap) | 2 X 1.0 mL @ 5 mg/mL (2 brown tubes, <u>black</u> caps) |
| Biotinylated Anti-hCOL1A1 stored in PBS, 0.1% Tween-20, 0.05% NaN ₃ , pH 7.4 | 20 µL @ 500 nM (1 tube, <u>black</u> cap) | 50 µL @ 500 nM (1 tube, <u>black</u> cap) | 500 µL @ 500 nM (1 tube, <u>black</u> cap) |
| hCOL1A1 Analyte* | 100 µL @ 3 µg/mL (1 tube, <u>clear</u> cap) | 100 µL @ 3 µg/mL (1 tube, <u>clear</u> cap) | 100 µL @ 3 µg/mL (1 tube, <u>clear</u> cap) |
| AlphaLISA Immunoassay Buffer (10X) ** | 2 mL, 1 small bottle | 10 mL, 1 small bottle | 100 mL, 1 large bottle |

* hCOL1A1 is supplied as a liquid at 3 µg/mL. The analyte can be stored at 4°C for up to 4 weeks or store long term at -20°C. Avoid multiple freeze-thaw cycles. One vial contains an amount of hCOL1A1 sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL353S).

** Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

*** The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 96- or 384-well assay plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Specific additional required reagents and materials:

The following materials are recommended:

| Item | Suggested source | Catalog # |
|----------------------------------|------------------|-----------|
| TopSeal™-A Adhesive Sealing Film | PerkinElmer Inc. | 6050185 |
| EnVision®-Alpha Reader | PerkinElmer Inc. | - |

Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H₂O (18 MΩ·cm) to dilute 10X AlphaLISA Immunoassay Buffer and to reconstitute the lyophilized analyte.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment. The standard curve should be performed in the AlphaLISA Immunoassay Buffer for serum and/or plasma samples.

Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols also include testing samples in 452 wells. If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

| Format | # of data points | Final | Volume | | | | Plate recommendation |
|---------|------------------|--------|--------|--------------------------|-----------------------|----------------|---|
| | | | Sample | AlphaLISA Acceptor beads | Biotinylated Antibody | SA-Donor beads | |
| AL371HV | 100 | 100 µL | 10 µL | 20 µL | 20 µL | 50 µL | White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560) |
| AL371C | 250 | 100 µL | 10 µL | 20 µL | 20 µL | 50 µL | White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560) |
| | 500 | 50 µL | 5 µL | 10 µL | 10 µL | 25 µL | White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350) |
| | 1 250 | 20 µL | 2 µL | 4 µL | 4 µL | 10 µL | Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290) |
| | 2 500 | 10 µL | 1 µL | 2 µL | 2 µL | 5 µL | Light gray AlphaPlate-1536 (cat # 6004350) |
| AL371F | 5 000 | 50 µL | 5 µL | 10 µL | 10 µL | 25 µL | White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350) |
| | 12 500 | 20 µL | 2 µL | 4 µL | 4 µL | 10 µL | Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290) |
| | 25 000 | 10 µL | 1 µL | 2 µL | 2 µL | 5 µL | Light gray AlphaPlate-1536 (cat # 6004350) |

3 Step Protocol described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). If a different amount of samples are tested, the volumes of all reagents have to be adjusted accordingly.

1) Preparation of 1X AlphaLISA Immunoassay Buffer:

- a. Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL H₂O.

2) Preparation of hCOL1A1 analyte standard dilutions:

- a. Take the hCOL1A1 analyte solution (100 µL @ 3 µg/mL) from the box.
 b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer (change tip between each standard dilution):

| Tube | Vol. of hCOL1A1 (µL) | Vol. of diluent (µL) * | [hCOL1A1] in standard curve | |
|-------------------|----------------------|------------------------|-----------------------------|-----------------|
| | | | (g/mL in 5 µL) | (pg/mL in 5 µL) |
| A | 10 µL of hCOL1A1 | 90 | 3.00E-07 | 300 000 |
| B | 60 µL of tube A | 120 | 1.00E-07 | 100 000 |
| C | 60 µL of tube B | 140 | 3.00E-08 | 30 000 |
| D | 60 µL of tube C | 120 | 1.00E-08 | 10 000 |
| E | 60 µL of tube D | 140 | 3.00E-09 | 3 000 |
| F | 60 µL of tube E | 120 | 1.00E-09 | 1 000 |
| G | 60 µL of tube F | 140 | 3.00E-10 | 300 |
| H | 60 µL of tube G | 120 | 1.00E-10 | 100 |
| I | 60 µL of tube H | 140 | 3.00E-11 | 30 |
| J | 60 µL of tube I | 120 | 1.00E-11 | 10 |
| K | 60 µL of tube J | 140 | 3.00E-12 | 3 |
| L | 60 µL of tube K | 120 | 1.00E-12 | 1 |
| M ** (background) | 0 | 100 | 0 | 0 |
| N ** (background) | 0 | 100 | 0 | 0 |
| O ** (background) | 0 | 100 | 0 | 0 |
| P ** (background) | 0 | 100 | 0 | 0 |

* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).

At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

** Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

3) Preparation of 5X AlphaLISA Anti-hCOL1A1 Acceptor beads (50 µg/mL):

- a. Prepare just before use.
 b. Add 50 µL of 5 mg/mL AlphaLISA Anti-hCOL1A1 Antibody Acceptor to 4950 µL of 1X AlphaLISA Immunoassay Buffer.

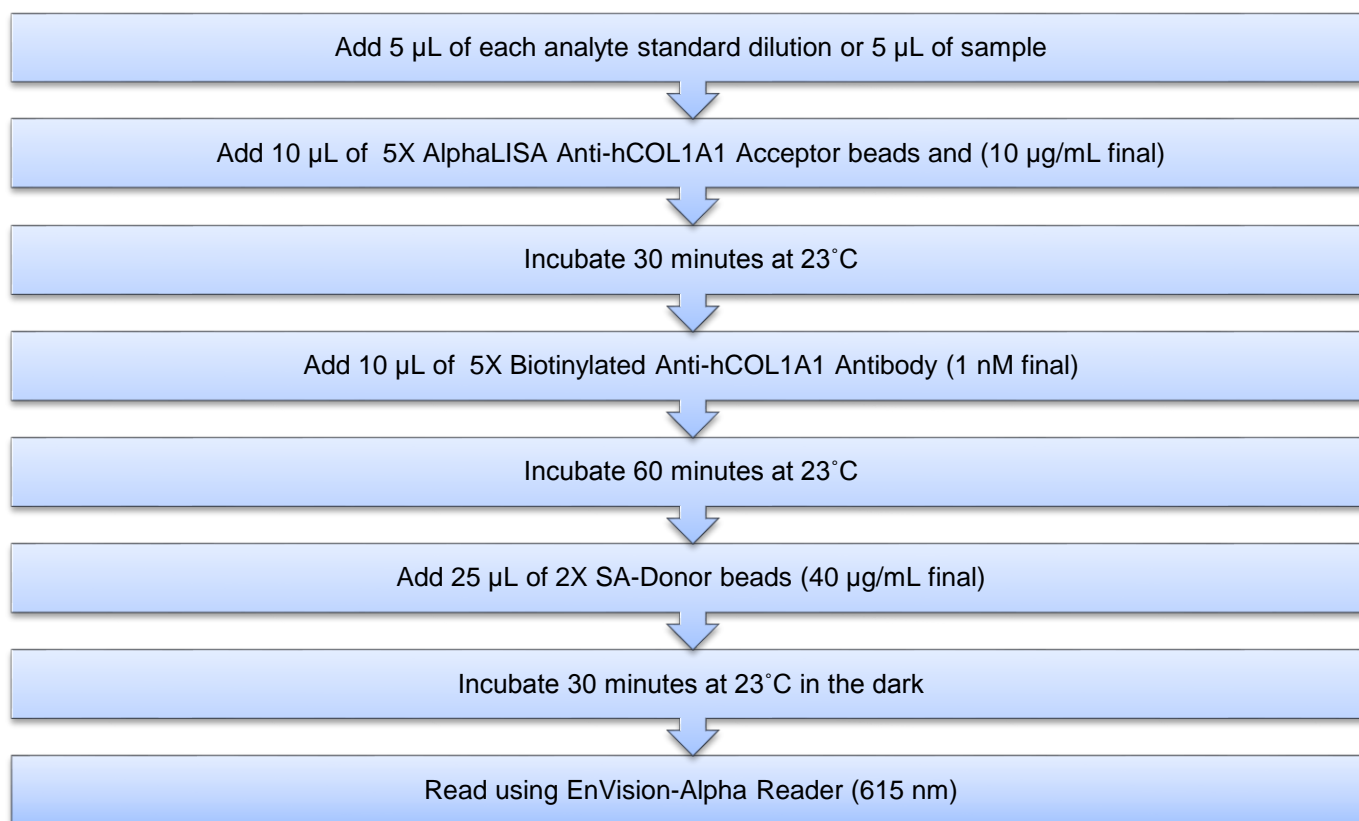
4) Preparation of 5X Biotinylated Anti-hCOL1A1 Antibody (5 nM):

- a. Prepare just before use.
 b. Add 50 µL of 500 nM Biotinylated Anti- hCOL1A1 Antibody to 4950 µL of 1X AlphaLISA Immunoassay Buffer.

5) Preparation of 2X Streptavidin (SA) Donor beads (80 µg/mL):

- a. Prepare just before use.
 b. Keep the beads under subdued laboratory lighting.
 c. Add 200 µL of 5 mg/mL SA-Donor beads to 12300 µL of 1X AlphaLISA Immunoassay Buffer.

6) In a white Optiplate (384 wells):



Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Performance Characteristics

AlphaLISA assay performance described below was determined using the 3 step protocol using AlphaLISA Immunoassay Buffer.

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5 μ L using the recommended assay conditions.

| LDL (pg/mL) | Buffer/Serum/Medium* | # of experiments |
|-------------|----------------------|------------------|
| 6 | IAB | 6 |
| 189 | DMEM | 6 |
| 264 | RPMI | 6 |

* The standard was prepared in these diluents. Note that LDL can be decreased (i.e. sensitivity increased) by preparing standards in different matrixes. Preparing standard in cell culture media with 10% FBS increases the background significantly.

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in IAB, DMEM, or RPMI. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well format using IAB.

- Intra-assay precision:

The intra-assay precision was determined using a total of 16 independent determinations in triplicate. Shown as CV%.

| hCOL1A1 | IAB | DMEM | RPMI |
|---------|-----|------|------|
| CV(%) | 6.0 | 5.4 | 6.3 |

- Inter-assay precision:

The inter-assay precision was determined using a total of 3 independent determinations with 9 measurements at 3 ng/mL. Shown as CV%.

| hCOL1A1 (3 ng/ml) | IAB | DMEM | RPMI |
|----------------------|-----|------|------|
| CV (%) | 15 | 10.9 | 13.6 |

- Spike Recovery:

Three known concentrations of analyte were spiked in IAB, or in cell culture media. All samples, including non-spiked buffer or media were measured in the assay. The average recovery from three independent measurements is reported. Note that the standard curves were prepared in IAB, DMEM, and RPMI. Note the cell culture media does not contain any FBS.

| Spiked hCOL1A1 (ng/mL) | % Recovery | | |
|------------------------|------------|------|------|
| | IAB | DMEM | RPMI |
| 100 | 101 | 103 | 89 |
| 10 | 98 | 93 | 104 |
| 1 | 99 | 100 | 106 |

- Specificity:

Cross-reactivity of the hCOL1A1 Kit was tested using the following proteins at 100 ng/mL in IAB.

| Protein | % Cross-reactivity |
|-------------------------|--------------------|
| Human Collagen Type II | 0 |
| Human Collagen Type III | 0 |
| Human Fibronectin | 0 |
| Human Hyaluronic Acid | 0 |

Human Serum Experiments

To validate the assay kit, commercially available human serum samples with unknown concentrations of hCOL1A1 was used to examine dilutional linearity. hCOL1A1 (~ 500 ng/mL) is detected in the human serum sample. A good dilutional linearity is observed when a sample is diluted 64 fold or greater.

| Dilution Factor | hCOL1A1 Detected (ng/mL) |
|-----------------|--------------------------|
| 1 | 20 |
| 2 | 51 |
| 4 | 107 |
| 8 | 184 |
| 16 | 288 |
| 32 | 363 |
| 64 | 482 |
| 128 | 467 |
| 256 | 533 |
| 512 | 491 |
| 1024 | 495 |

| | |
|------|-----|
| 2048 | 459 |
|------|-----|

Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

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