ELISA Kit for Measuring Human Lipocalin-1

CircuLex Human Lipocalin-1 ELISA Kit

Cat# CY-8110

Intended Use

The MBL Research Product **CircuLex Human Lipocalin-1 ELISA Kit** is used for the quantitative measurement of human lipocalin-1 in tear fluid.

Individual users should determine appropriate conditions when using other types of samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Do not expose reagents to excessive light.

Introduction

Lipocalin-1, also called tear lipocalin or von Ebner's gland protein (1, 2), a member of the lipocalin superfamily that binds to a broad array of different chemical classes of lipophilic ligands (2-4), is believed to act as a physiological scavenger of putative harmful lipophilic ligands (1, 4). Thus, it was thought to be produced exclusively by a number of exocrine glands and tissues, including lachrymal and lingual glands, prostate, secretory glands of the tracheobronchial tract, pituitary gland and sweat glands (1, 5). It was also demonstrated to exhibit cysteine proteinase inhibition and nonspecific endonuclease activity in vitro (6-8).

Lipocalin-1 concentration in tear fluid is decreased in patients with meibomian gland dysfunction (9). It was identified the first protein, lipocalin-1, in the secretome of human blastocysts that is associated with chromosome aneuploidy thus lipocalin-1 might be a potential marker for noninvasive aneuploidy screening (10). Lipocalin-1 secretion is up-regulated in the airways of patients with cystic fibrosis (11), by contrast, down-regulated in in dry eye syndrome, accompanying with decrease of prolactin-inducible protein, lactoferrin and lysozyme (12). Furthermore, lipocalin-1 was identified as a novel autoantigen target in Sjögren's syndrome (13). The major tear proteins; such as lipocalin-1, lactoferrin and lysozyme are involved in the immune and inflammatory processes and defense against pathogens (14).

Principle of the Assay

The MBL Research Product **CircuLex Human Lipocalin-1 ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human lipocalin-1 is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human lipocalin-1 present. After washing away any unbound substances, an HRP conjugated antibody specific for human lipocalin-1 is added to the wells. Following a wash to remove any unbound antibody HRP conjugate, the remaining conjugate is allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of human lipocalin-1. A standard curve is constructed by plotting absorbance values versus human lipocalin-1 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Summary of Procedure

Add 100 µL of diluted samples to the wells
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of HRP conjugated anti-human lipocalin-1 antibody
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓ Incubate for 10-20 minutes at room temp.
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with anti-human lipocalin-1 antibody as a capture antibody.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing Tween®-20.

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for reconstitution of Human Lipocalin-1 Standard and sample dilution. Ready to use.

Human Lipocalin-1 Standard: One vial containing X* ng of lyophilized recombinant human lipocalin-1.
***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**
20X HRP conjugated Detection Antibody: One bottle containing 600 µL of HRP (horseradish peroxidase) conjugated anti-human lipocalin-1 antibody.

Conjugate Dilution Buffer: One bottle containing 12 mL of Conjugate Dilution Buffer.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H_2SO_4. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**
- **500 or 1,000 mL graduated cylinder**.
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human origin, and these reagents. In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Sample Collection and Storage

Tear fluid: Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles.

Preparation of tear extract from Schirmer's test strip:

1. Place one free end of the Schirmer's test strip within your lower eyelid. Both eyes are tested at the same time.

2. Keep your eyes gently closed for 5 minutes.

3. Remove the wet test strips from each lower eyelid.

4. Cut the wet test strip 5 mm length with scissors or cutter.

6. Transfer 5 mm piece of the wet test strip to microcentrifuge tube.

7. Add 300 µl PBS with protease inhibitor cocktail to the microcentrifuge tube, incubated at 4°C for 3 hours with intermittent mixing.

8. Centrifuge at 15,000 rpm for 5 minutes at 4°C.

9. Transfer the supernatant to new microcentrifuge tube, store at -70°C until processing*.

   * The tear extract from Schirmer's test strip can be stored at below -70°C. Avoid multiple freeze/thaw cycles. After thaw the tear extract, centrifuge at 15,000 rpm for 5 minutes at 4°C again since the cell lysates should be clear of any sediments or particulate matter.

10. This tear extract from Schirmer's test strip may require 100- to 300-fold dilution for Assay**.

    ** Typical data using this protocol are shown in Fig. 1 in the section "Example of Test Results" below.

Detailed Protocol

The MBL Research Product **CircuLex Human Lipocalin-1 ELISA Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the Human Lipocalin-1 Standard within the kit should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer**, **20X HRP-conjugated Detection Antibody** and **Human Lipocalin-1 Standard**.

1. Prepare a working solution of Wash Buffer by adding 100 mL of the **10X Wash Buffer** to 900 mL of deionized (distilled) water. Mix well. Store at 4°C for two weeks or -20°C for long-term storage.

2. Prepare **1X HRP conjugated Detection Antibody** by 20-fold diluting **20X HRP-conjugated Detection Antibody** with Conjugate Dilution Buffer at the time of use and discard any unused portion after use.

3. Reconstitute **Human Lipocalin-1 Standard** with X* **µL** of **Dilution Buffer** by gently mixing. After reconstitution, immediately dispense it in small aliquots (e.g. 100 µL) to plastic micro-centrifuge tubes and store below -70°C to avoid non-specific adsorption to glass surface and multiple freeze-thaw cycles. The concentration of the reconstituted Human Lipocalin-1 Standard should be **512 ng/mL**, which is referred to as the **Master Standard** of human lipocalin-1.
***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**
Prepare Standard Solutions as follows:
Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. **Std.1 (128 ng/mL)** serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.1	150 µL of Master Standard (512 ng/mL)	450 µL	128 ng/mL
Std.2	300 µL of Std. 1 (128 ng/mL)	300 µL	64 ng/mL
Std.3	300 µL of Std. 2 (64 ng/mL)	300 µL	32 ng/mL
Std.4	300 µL of Std. 3 (32 ng/mL)	300 µL	16 ng/mL
Std.5	300 µL of Std. 4 (16 ng/mL)	300 µL	8 ng/mL
Std.6	300 µL of Std. 5 (8 ng/mL)	300 µL	4 ng/mL
Std.7	300 µL of Std. 6 (4 ng/mL)	300 µL	2 ng/mL
Blank	-	300 µL	0 ng/mL

Note: Do not use a Repeating pipette. Change tips for every dilution. Wet tip with Dilution Buffer before dispensing.

Sample Preparation

Dilute samples with **Dilution Buffer**.

• Tear fluids may require 50,000- to 100,000-fold dilution.

Assay Procedure

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Dilute samples with **Dilution Buffer**. (See "Sample Preparation" above.)

3. Pipette **100 µL** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.

4. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

5. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

6. Add **100 µL** of **1X HRP conjugated Detection Antibody** into each well.

7. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

8. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

9. Add **100 µL** of **Substrate Reagent**. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended. Return Substrate Reagent to 4°C immediately after the necessary volume is removed

10. Incubate the plate **at room temperature (ca.25°C) for 10-20 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. The incubation time may be extended up to 30 minutes if the reaction temperature is below 20°C.

11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Note-2: Reliable standard curves are obtained when either O.D. values do not exceed 0.25 units for the blank (zero concentration), or 3.0 units for the highest standard concentration. The plate should be monitored at 5-minute intervals for approximately 30 minutes.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine the concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculations

Average the duplicate readings for each standard, control and sample, and subtract the optical density of the average zero standard. Plot the optical density versus the concentration of standards and draw the best curve. Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve fits best to a sigmoidal four-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a four-parameter logistic function.

A standard curve is also to be constructed by plotting the absorbance (Y) versus log of the known concentration (X) of standards, using a cubic function. Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of optical density (Y) is plotted versus log of the known concentration (X) of standards). To determine the concentration of each sample, first find the optical density on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Measurement Range

The measurement range is 2 ng/mL to 128 ng/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human lipocalin-1 concentration.

Troubleshooting

1. All samples and standards should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.

3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **CircuLex Human Lipocalin-1 ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date.

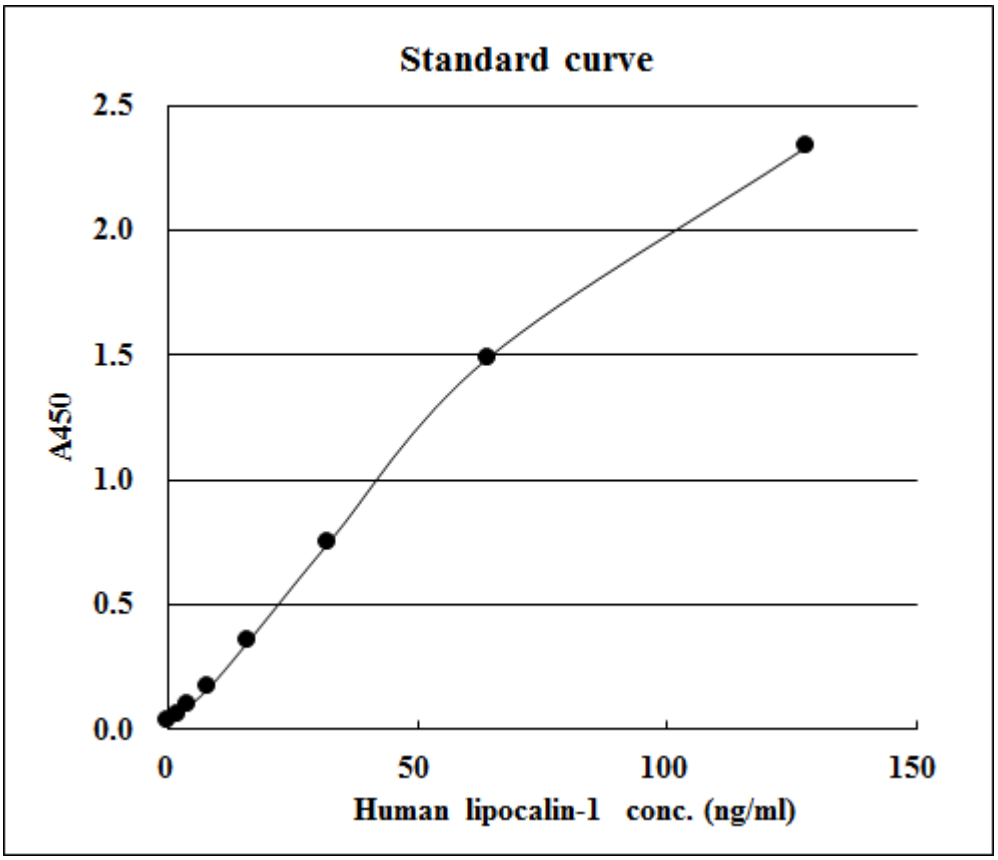
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of human lipocalin-1 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 0.96 ng/mL of sample.

* Dilution Buffer was pipetted into blank wells.

Typical Standard Curve



2. Precision

Intra-assay Precision (Precision within an assay)
Three samples* of known concentration were tested sixteen times on one plate to assess intra-assay precision.

• Intra-assay (Within-Run, n=16) CV=5.3-8.6%

* Sample: Tear fluid

Human lipocalin-1 conc. (mg/mL)

	Sample 1	Sample 2	Sample 3
1	1.18	3.84	6.23
2	1.04	3.52	5.06
3	1.22	3.68	5.96
4	1.17	3.68	6.32
5	1.26	3.76	6.04
6	1.02	3.26	5.61
7	1.26	3.84	5.82
8	1.24	3.84	6.01
9	1.21	3.64	5.97
10	1.06	3.42	4.67
11	1.26	3.58	6.38
12	1.13	3.46	5.42
13	1.20	3.55	5.34
14	0.99	3.20	5.81
15	1.20	3.56	5.70
16	1.34	3.61	5.59
MAX.	1.34	3.84	6.38
MIN.	0.99	3.20	4.67
MEAN	1.17	3.59	5.75
S.D.	0.10	0.19	0.46
C.V.	**8.6%**	**5.3%**	**8.0%**

Inter-assay Precision (Precision between assays)

Three samples* of known concentration were tested in four separate assays to assess inter-assay precision.

- Inter-assay (Run-to-Run, n=4) CV=1.6-5.6%

* Sample: Tear fluid

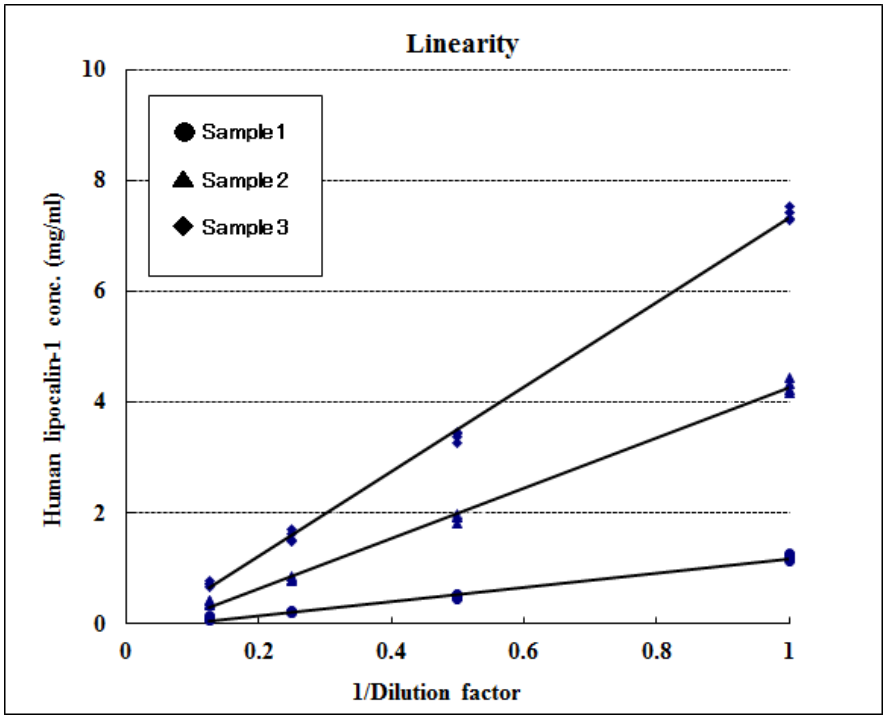
Human lipocalin-1 conc. (mg/mL)

	Sample 1	Sample 2	Sample 3
1	1.36	3.71	6.66
2	1.22	3.59	7.19
3	1.22	3.61	6.66
4	1.22	3.59	7.19
MAX.	1.36	3.71	7.19
MIN.	1.22	3.59	6.66
MEAN	1.26	3.63	6.93
S.D.	0.071	0.058	0.307
C.V.	**5.6%**	**1.6%**	**4.4%**

3. Linearity

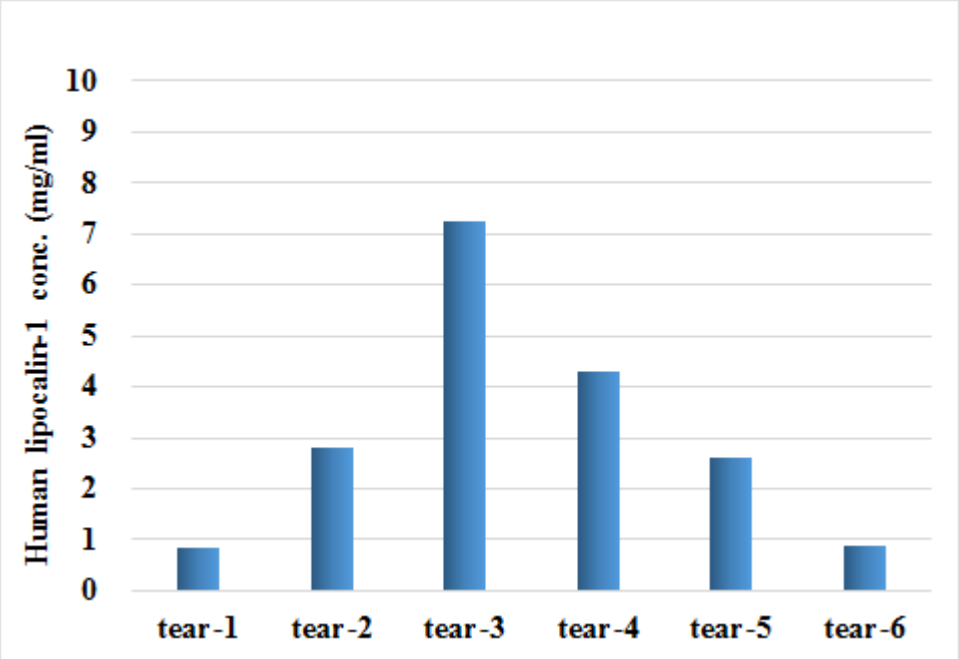
Three samples* were diluted with Dilution Buffer and assayed after dilution. The 10^5-fold diluted tear samples were set to 1.

* Sample: Tear fluid



Example of Test Results

Fig.1 Human lipocalin-1 concentration in tear fluids from six healthy volunteers



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Related Products

*** CircuLex S100A4 ELISA Kit Ver.2: Cat# CY-8086**
*** CircuLex S100A6 ELISA Kit: Cat# CY-8097**
*** CircuLex S100A10 ELISA Kit: Cat# CY-8095**
*** CircuLex S100A11 ELISA Kit: Cat# CY-8063**
*** CircuLex S100A14 ELISA Kit: Cat# CY-8064**
*** CircuLex S100P ELISA Kit: Cat# CY-8060**

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