



# **ANXA1 Chemi-Luminescent ELISA Kit (Human) (OKCD03297) Instructions for use**

For the quantitative measurement of ANXA1 in serum, plasma and other biological fluids.

This product is intended for research use only.

Lot to lot kit variations can occur. Refer to the manual which has been provided with the kit.

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## 1. Background

### Principle

Aviva Systems Biology ANXA1 Chemi-Luminescent ELISA Kit (Human) (OKCD03297) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for ANXA1 has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for ANXA1 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of a luminol substrate which is catalyzed by the HRP to produce light emission. The light emission is read by a luminometer (or photo-multiplier equipped instrument) and the intensity of the emitted light is proportional to the amount of sample ANXA1 captured in well.

### Background

Plays important roles in the innate immune response as effector of glucocorticoid-mediated responses and regulator of the inflammatory process. Has anti-inflammatory activity . Plays a role in glucocorticoid-mediated down-regulation of the early phase of the inflammatory response . Promotes resolution of inflammation and wound healing . Functions at least in part by activating the formyl peptide receptors and downstream signaling cascades . Promotes chemotaxis of granulocytes and monocytes via activation of the formyl peptide receptors . Contributes to the adaptive immune response by enhancing signaling cascades that are triggered by T-cell activation, regulates differentiation and proliferation of activated T-cells . Promotes the differentiation of T-cells into Th1 cells and negatively regulates differentiation into Th2 cells . Has no effect on unstimulated T cells . Promotes rearrangement of the actin cytoskeleton, cell polarization and cell migration . Negatively regulates hormone exocytosis via activation of the formyl peptide receptors and reorganization of the actin cytoskeleton . Has high affinity for Ca<sup>2+</sup> and can bind up to eight Ca<sup>2+</sup> ions . Displays Ca<sup>2+</sup>-dependent binding to phospholipid membranes . Plays a role in the formation of phagocytic cups and phagosomes. Plays a role in phagocytosis by mediating the Ca<sup>2+</sup>-dependent interaction between phagosomes and the actin cytoskeleton .

### General Specifications

| General Specifications |                                                                                                                                                                                                                                |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Range                  | 3.12 - 200 ng/mL                                                                                                                                                                                                               |
| LOD                    | < 1.08 ng/mL (Derived by linear regression of OD <sub>450</sub> of the Mean Blank + 2xSD)                                                                                                                                      |
| Specificity            | Human Annexin A1<br>UniProt ID: P04083<br>GeneID: 301<br><u>Target Alias</u> : Annexin-1, Annexin A1, Annexin I, ANX1, Calpactin-2, Calpactin II, Chromobindin-9, Lipocortin I, LPC1, p35, Phospholipase A2 inhibitory protein |
| Cross-Reactivity       | No detectable cross-reactivity with other relevant proteins                                                                                                                                                                    |

## 2. Assay Summary



## 3. Storage and Stability

- Upon receipt store kit at 4°C for 1 month or -20°C for 6 months, noted exceptions below. Do not use past expiration date.

## 4. Kit Components

- The following reagents are the provided contents of the kit.

| Description                               | Quantity                      | Storage Conditions                    |
|-------------------------------------------|-------------------------------|---------------------------------------|
| Anti-ANXA1 Microplate                     | 96 Wells (12 x 8 Well strips) | 4°C for 1 Month or -20°C for 6 Months |
| ANXA1 Lyophilized Standard                | 2 x 200 ng                    |                                       |
| 100X Biotinylated ANXA1 Detector Antibody | 1 x 120 µL                    |                                       |
| 100X Avidin-HRP Conjugate                 | 1 x 120 µL                    |                                       |
| Standard Diluent                          | 1 x 20 mL                     | 4°C for 6 Month                       |
| Detector Antibody Diluent                 | 1 x 12 mL                     |                                       |
| Conjugate Diluent                         | 1 x 12 mL                     |                                       |
| 30X Wash Buffer                           | 1 x 20 mL                     |                                       |
| 100X Luminol Substrate                    | 1 x 2 mL                      |                                       |
| Substrate Diluent                         | 1 x 20 mL                     |                                       |

## 5. Precautions

- Read instructions fully prior to beginning use of the assay kit.

- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

## 6. Required Materials Not Supplied

- Luminometer or photo-multiplier tube (PMT) equipped microplate reader capable of the following parameters: lag time 30.0 seconds, read time 1.0 seconds per well.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5  $\mu$ L through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator (optional)

## 7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting light intensity measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1  $\mu$ L is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- Luminol Substrate is easily contaminated and labile. Handle carefully and protect from light.