



# **NLRP3 Chemi-Luminescent ELISA Kit (Human) (OKCD03830) Instructions for use**

For the quantitative measurement of NLRP3 in tissue homogenates, cell lysates 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 NLRP3 Chemi-Luminescent ELISA Kit (Human) (OKCD03830) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for NLRP3 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 NLRP3 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 NLRP3 captured in well.

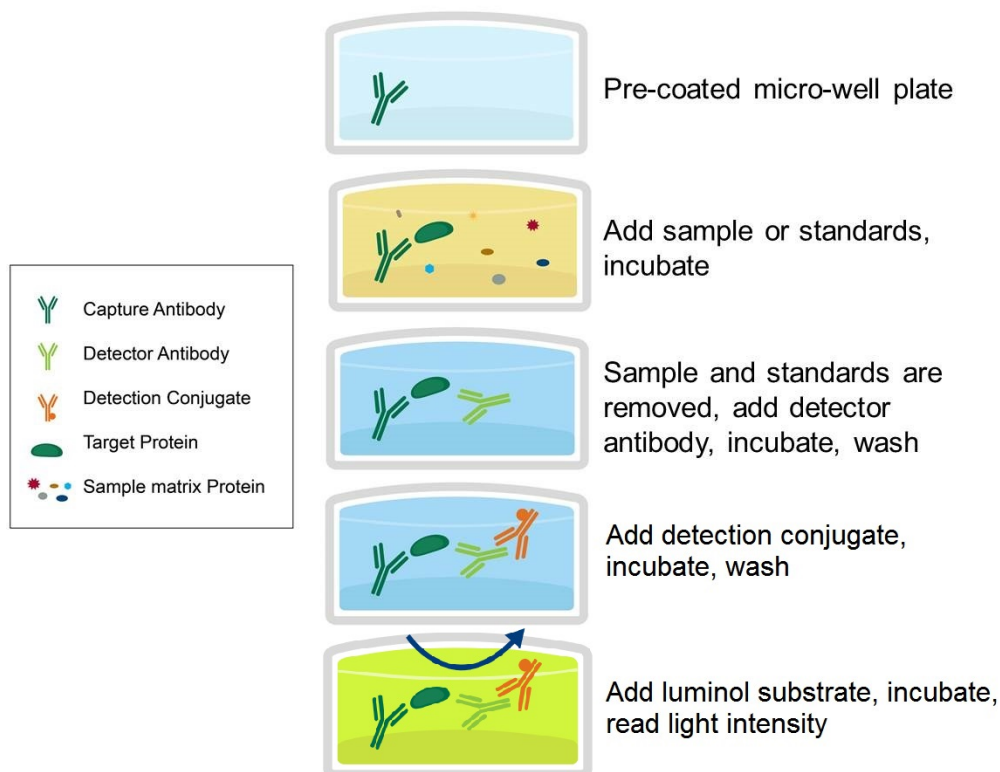
### Background

As the sensor component of the NLRP3 inflammasome, plays a crucial role in innate immunity and inflammation. In response to pathogens and other damage-associated signals, initiates the formation of the inflammasome polymeric complex, made of NLRP3, PYCARD and CASP1 (and possibly CASP4 and CASP5). Recruitment of proCASP1 to the inflammasome promotes its activation and CASP1-catalyzed IL1 $\beta$  and IL18 maturation and secretion in the extracellular milieu. Activation of NLRP3 inflammasome is also required for HMGB1 secretion. The active cytokines and HMGB1 stimulate inflammatory responses. Inflammasomes can also induce pyroptosis, an inflammatory form of programmed cell death. Under resting conditions, NLRP3 is autoinhibited. NLRP3 activation stimuli include extracellular ATP, reactive oxygen species, K<sup>+</sup> efflux, crystals of monosodium urate or cholesterol, beta-amyloid fibers, environmental or industrial particles and nanoparticles, etc. However, it is unclear what constitutes the direct NLRP3 activator. Independently of inflammasome activation, regulates the differentiation of T helper 2 (Th2) cells and has a role in Th2 cell-dependent asthma and tumor growth. During Th2 differentiation, required for optimal IRF4 binding to IL4 promoter and for IRF4-dependent IL4 transcription. Binds to the consensus DNA sequence 5'-GRRGGNRGAG-3'. May also participate in the transcription of IL5, IL13, GATA3, CCR3, CCR4 and MAF.

### General Specifications

General Specifications	
Range	0.0686 - 50 ng/mL
LOD	< 27.5 pg/mL (Derived by linear regression of OD <sub>450</sub> of the Mean Blank + 2xSD)
Specificity	Human NACHT, LRR and PYD domains-containing protein 3 UniProt ID: Q96P20 GenID: 114548 Target Alias: AGTAVPRL, AII, AII/AVP, Angiotensin/vasopressin receptor AII/AVP-like, AVP, C1orf7, Caterpillar protein 1.1, CIAS1, CLR1.1, Cold autoinflammatory syndrome 1 protein, Cryopyrin, FCAS, FCU, FLJ95925, MWS, NACHT, LRR and PYD domains-containing protein 3, NAL
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-NLRP3 Microplate	96 Wells (12 x 8 Well strips)	4°C for 1 Month or -20°C for 6 Months
NLRP3 Lyophilized Standard	2 x 50 ng	
100X Biotinylated NLRP3 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.