NLRP3 Inflammasome Human Reagents Starter Set

Prod. No. AG-44B-0010-KI01 *For Research Use Only* *June-15-2021*

1. Description and Intended Use

The NLRP3 Inflammasome Human Reagents Starter Set is an all-in-one product to study the NLRP3 inflammasome using Western blotting application. This starter set comprises our KO extract validated STANDARD antibodies against the key components of the NLRP3 inflammasome including NLRP3, Asc and Caspase-1, used and published by the experts in inflammasome research. It also contains two chemical compounds for priming and activation of the NLRP3 inflammasome. This economic starter set contains enough primary antibodies to perform at least 3 western blot experiments.

2. This Set Contains

• AG-20B-0014	anti-NLRP3/NALP3, mAb (Cryo-2)	Mouse IgG2b	25 µg
• AG-20B-0048	anti-Caspase-1 (p20) (human), mAb (Bally-1)	Mouse IgG1	25 µg
• AG-25B-0006	anti-Asc, pAb (AL177)	Rabbit IgG	25 µg
• IAX-100-007	LPS from E. coli R515 (Re) TLRpure Sterile Solution		500 µg
• AG-CN2-0020	Nigericin . sodium salt		5 mg

NOTE: *All antibodies are supplied at a concentration of 0.5mg/ml in PBS, containing a preservative. Nigericin is provided as solid powder. These components are provided in a box and can be stored for ≥12 months after receipt at -20°C.The LPS is provided in a Ready-to-Use solution in a separate container together with the box and should be stored at 4°C after receipt. Freeze/thaw cycles for the LPS should be avoided.*

3. Product Specifications

Each antibody in the NLRP3 Inflammasome Human Reagents Starter Set has been thoroughly validated in KO experimental setup and detects endogenous levels of its target protein.

- anti-NLRP3/NALP3, mAb (Cryo-2) (#AG-20B-0014) detects endogenous levels of full-length human and mouse NLRP3 (~120kDa). This antibody has been produced by using recombinant mouse NLRP3/NALP3 (pyrin domain/aa 1-93) as an immunogen.
- anti-Caspase-1 (p20) (human), mAb (Bally-1) (#AG-20B-0048) detects specifically endogenous levels of full-length human caspase-1 (~50kDa), the cleaved p20 subunit (~20kDa) and also other caspase-1 cleavage bands, such as the p33 (part of the active caspase-1, ref. 1), which are specific based on KO validation experiments. This antibody has been produced by using the recombinant human caspase-1 as an immunogen.
- anti-Asc, pAb (AL177) (#AG-25B-0006) detects endogenous levels of full-length human and mouse Asc (~25kDa) and a splice variant of Asc (~18kDa). This antibody has been produced by using a synthetic peptide corresponding to a region at the N-terminal human Asc as an immunogen.

Both chemicals LPS and Nigericin in the NLRP3 Inflammasome Human Reagents Starter Set are required for priming (Signal 1) and activation (Signal 2) of the NLRP3 inflammasome, respectively.

- LPS from E. coli R515 (Re) TLRpure Sterile Solution (#IAX-100-007) is a PAMP (Pathogen-Associated Molecular Pattern) from Gram-negative bacteria that binds to the TLR4 receptor to trigger the first step (priming reaction, Signal 1) of the inflammasome activation. It can be used at 50 ng/ml for 3-4 hours.

- Nigericin . sodium salt (#AG-CN2-0020) is a potassium efflux activator that triggers the activation step of inflammasomes (Signal 2). It can be used at 50 µM for 30 minutes.

NOTE: *For further information including applications, please refer to primary antibody datasheets or product webpages.*

Reference 1: Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity: D. Boucher, et al.; J. Exp. Med. **215,** 827 (2018)

4. Materials Required for Western Blotting but not provided

- Phosphate Buffered Saline (PBS)
- Tris Buffered Saline (TBS)
- SDS Sample Buffer
- Tris-Glycine SDS Running Buffer
- Tris-Glycine Transfer Buffer
- Tris Buffered Saline with Tween 20 (TBST)
- Non-fat Dry Milk
- Bovine Serum Albumin (BSA)

- Wash Buffer: 1X TBST (TBS 1X + 0.1% Tween 20).
- Blocking Buffer: 1X TBST with 5% w/v non-fat dry milk; for 150 ml, add 7.5 g non-fat dry milk to 150 ml 1X TBST and mix well.
- Primary Antibody Dilution Buffer: 1X TBST with 5% BSA or 5% non-fat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or non-fat dry milk to 20 ml 1X TBST and mix well.
- Pre-stained Protein Marker, Broad Range (Premixed Format).
- Blotting Membrane and Paper: The below-mentioned protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP: anti-mouse IgG-HRP (#AG-29B-0004E); anti-rabbit IgG HRP (#AG-29B-0006E).
- Chemiluminescent Detection Reagent/System.

5. General Western Blot Protocol

For western blot, incubate membrane with diluted primary antibody (1 µg/ml) in 1X TBST containing either 5% w/v BSA or non-fat dry milk at 4°C with gentle shaking, overnight.

Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing reagents for priming and activation of inflammasomes (see section 3) for the desired time.
2. After incubation, aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. *Optional: Sonicate for 10-15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).*
5. Heat a 30 µl sample to 95-100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). NOTE: Loading of pre-stained molecular weight markers to verify electrotransfer and to determine molecular weights is recommended.
8. Electrotransfer to nitrocellulose membrane using a liquid device (see section 6 for NLRP3 transfer).

Membrane Blocking and Antibody Incubation

NOTE: *Volumes are for 10 cm x 10 cm (100 cm2) of membrane; for different sized membranes, adjust volumes accordingly.*

i. Membrane Blocking

1. After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature. (Optional)
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

ii. Primary Antibody Incubation

1. Incubate membrane and primary antibody (1 µg/ml) in 8 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate and freshly prepared HRP-conjugated secondary antibody (at 1:5000) in 10 ml of blocking buffer with gentle agitation for 1 hr at RT.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection.

Detection of Proteins

1. Incubate membrane with detection chemiluminescent reagent.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.

NOTE: *Due to the kinetics of the detection reaction, signal is intense immediately following incubation and declines over the following 2 hr.*

6. Technical Hints & Tips for NLRP3 Western Blotting

i) Cells or Tissues:

- Verify in the literature that primary cells, tissues or cell lines that are used, express NLRP3 at detectable levels. Priming with LPS, PMA or other priming reagents (that activate NF-κB signaling) can be performed to increase NLRP3 levels.

- NLRP3 is expressed in many different cells or tissues, but in some cases (e.g. fortuitous mutations in some cell subclones), levels of NLRP3 can be dysregulated leading to no or weaker signal in WB.

ii) SDS-PAGE Gel:

- Percentage SDS-PAGE should be optimally <8%.
- All buffers (running, stacking, transfer) should be made fresh.
- Load at least 20-30µg per lane.
- NLRP3 transfer is tricky. It should be performed in a wet tank device overnight at 20V at 4°C.
- Transfer for 1 hr and dry or semi-dry devices is **NOT** recommended.

iii) Primary Antibody:

- Use freshly prepared antibody anti-NLRP3 (Cryo-2) at 1 µg/ml. If protein is weakly expressed, higher concentration, such as 2 µg/ml can be used.

v) Positive & Negative Controls:

- As positive control cells (that can be primed with ATP, PMA or LPS to increase NLRP3 levels), we recommend human monocytic cell lines THP1 or U937
- As a negative control cell line, we recommend human HEK 293.

7. Useful Resources

Caspase-1:

- Measuring the Inflammasome (Download Protocol)
- Immunoblotting for Active Caspase-1 (Download Protocol)

NLRP3:

- Measuring NLR Oligomerization I (Download Protocol)

Asc:

- Measuring Inflammasome Activation in Response to Bacterial Infection (Download Protocol)
- Cell-Free Assay for Inflammasome Activation (Download Protocol)

8. Other Reagents Useful for Inflammasome Research

***Inflammasome Activation**:*

- Monosodium urate (ready-to-use) (AG-CR1-3951) *(Use at 150 µg/ml for 6 hours)*

Inflammasome Inhibition:

- MCC950 . sodium salt (AG-CR1-3615) *(Use at 0.1 to 1µM)*

Other Inflammasome Starter Sets and Kits available from AdipoGen Life Sciences:

NLRP3 Inflammasome Mouse Antibodies Starter Set (AG-44B-0009)

NLRP3 Inflammasome Mouse Reagents Starter Set (AG-44B-0011)

For Additional Inflammasome Product Information:

https://adipogen.com/inflammasomes
https://adipogen.com/gasdermin-d
https://adipogen.com/panoptosis

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