

Western Blotting Protocol

Product Information

Product Name: Catalog Number: anti-NLRP3 /NALP3, mAb (Cryo-2) AG-20B-0014

NLRP3/NALP3 Detection by Western Blotting:

1. Priming (key step for NLRP3/NALP3 detection by Western blotting):

Human: THP1 cells are cultured in RPMI 1640 media, supplemented with 10% FBS. Cells are treated for 3h with phorbol-12-myristate-13-acetate (PMA) (100nM) (#AG-CN2-0010) or lipopolysaccharide (LPS) (50ng/ml) (#AG-CU1-0001).

Murine: Bone marrow-derived dendritic cells (BMDCs) or bone marrow-derived macrophages (BMDMs) are cultured in RPMI 1640 media, supplemented with 10% FBS. Cells are treated for 3h with lipopolysaccharide (LPS) (50ng/ml) (#AG-CU1-0001).

During this step, NLRP3/NALP3 expression is increased.

2. Inflammasome activation (optional for NLRP3/NALP3 detection):

Human PMA or LPS-differentiated THP1 cells Murine LPS-primed BMDCs or BMDMs

are plated in 12-well plates and treated for 6h with monosodium urate (MSU) (150µg/ml) (#AG-CR1-3951) or for 30min with Nigericin (5µM) (#AG-CN2-0020).

3. Western blotting:

Cell extracts from activated cells are processed for immunoblotting, separated on a 7.5 % SDS-PAGE gel and electrotransferred to nitrocellulose or PVDF membrane.

- Human/mouse NLRP3 is detected by NLRP3 /NALP3, mAb (Cryo-2) (1µg/ml) (#AG-20B-0014)
- Mouse NLRP3 is detected by NLRP3 /NALP3 (mouse), mAb (Cryo-1) (1µg/ml) (#AG-20B-0006)

23-10-2018/AT

WARNING: Intended for research use only. This product is not intended or approved for human, diagnostics, therapeutic or veterinary use. Use of this product for human or animal testing is extremely hazardous and may result in disease, severe injury, or death. MATERIAL SAFETY DATA: Review the complete Material Safety Data Sheet before use.

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