

Product Information

Product Name: anti-Asc, pAb (AL177)
Catalog Number: AG-25B-0006

Cell culture:

- THP-1 cells are cultured in RPMI 1640, supplemented with 10% heat inactivated fetal bovine serum (FCS), 50 µM β-mercaptoethanol, and penicillin/streptomycin (100µg/ml of each).
- THP-1 cells are grown in suspension to a density of $\sim 1.5 \times 10^6$ cells/ml and are pre-stimulated with LPS (1µg/ml) or PBS (control) for 1 hr.

Cells extracts preparation:

- Cytosolic extracts are prepared from stimulated or unstimulated THP-1 cells as follows: the cells are harvested and washed in cold phosphate-buffered saline. This is followed by swelling in 5 volumes of ice-cold hypotonic buffer W (20mM HEPES-KOH [pH 7.5], 10mM KCl, 1.5mM MgCl₂, 1mM Na EDTA, 1mM Na EGTA, 0.1mM PMSF, and the Roche protease inhibitor cocktail) and incubation on ice for 15 min.
- The cellular membrane integrity is then disrupted by passage 15 times through a G22 needle. Cell lysates are centrifuged, and the supernatants, following filtration (0.45 µM), are used for this *in vitro* Caspase-1 cleavage assay.

In vitro Inflammasome (Caspase-1 cleavage) activation:

- Following incubation at 30°C (5, 10, 30 and 60 min) of the extracts, caspase-1 cleavage is monitored by Western analysis with the specific human caspase-1 antibody (Bally-1) (Adipogen, #AG-20B-0048).

Asc, pAb (AL177) functional assay (inhibition):

- To test the requirement of the protein Asc in this cell-free assay, 5µg of Asc, pAb (AL177) (#AG-25B-0006) is added to the prepared supernatants (about 500µg/reaction) for 5 min prior to incubation at 30°C and inflammasome activation. The activity of the Asc, pAb (AL177) antibody is blocked by pre-incubation with its blocking peptide (Adipogen, #AG-37B-0001).

04-10-2018/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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