

Data Sheet

MOUSE ANNEXIN V–Alexa488

RECOMBINANT

Catalog no.:	AP1011AG.1 / AP1011AG.2
Name:	Annexin V conjugated with Alexa-Fluor 488
Synonyms:	Annexin-5, Lipocortin V, Endonexin II, Calphobindin I (CBP-I), Placental anticoagulant protein I (PAP-I), Placental anticoagulant protein 4 (PP4), Thromboplastin inhibitor, Vascular anticoagulant-alpha (VAC-alpha), Anchorin CII
Conjugate:	Alexa Fluor 488
	Maximum of Absorption at 495 nm, Maximum of Emission at 519 nm
Swiss-Prot No:	P48036
Gene Information:	Gene Name: Anxa5, Anx5
	GeneID: 11747
Source:	*E. coli*
Purification:	Affinity chromatography, SDS-PAGE
Storage buffer:	PBS, 0.02 % NaN_3
Identity:	Mass spectrometry peptide mass fingerprinting
Specificity:	Binds specific to phosphatidylserine (PS) in the presence of calcium[2]
Applications:	Annexin V is used for the detection of apoptosis in mouse and humans (not tested in other species).
	Apoptosis is characterised by the loss of membrane asymmetry. In healthy cells, PS is located on the inner leaflet of the cell membrane whereas in apoptotic cells PS is found on the outer leaflet of the membrane. Hence, apoptotic events can be detected by binding of AnnnexinV-Alexa488 to the exposed PS. Since AnnexinV can pass the membrane of necrotic cells, counterstaining with propidium iodide is recommended to distinguish necrotic and apoptotic cells.
Contents:	50 µl / 500 µl (5 µl are suitable for one assay)
Store at:	- 20°C

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Staining protocol:

1. Harvest 2x10^5 cells per staining and wash cells twice in cold phosphate-buffered-saline (PBS)

2. Resuspend cells in 100 µl binding buffer, add 5µl annexin V-Alexa488 to the cells and mix gently.

For detection of necrosis add additionally appropriate amounts of propidium iodide (final concentration 1µg/ml or 1.5µM).

3. Incubate for 15min at room temperature in the dark

4. Add 400 µl binding buffer and analyse the staining in flow cytometry as soon as possible (within an hour).

Material not provided:

Binding Buffer:	10 mM Hepes pH7.4, 140 mM NaCl, 2.5 mM $CaCl_2$
Propidium iodide:	50 µg/ml, final concentration 1 µg/ml

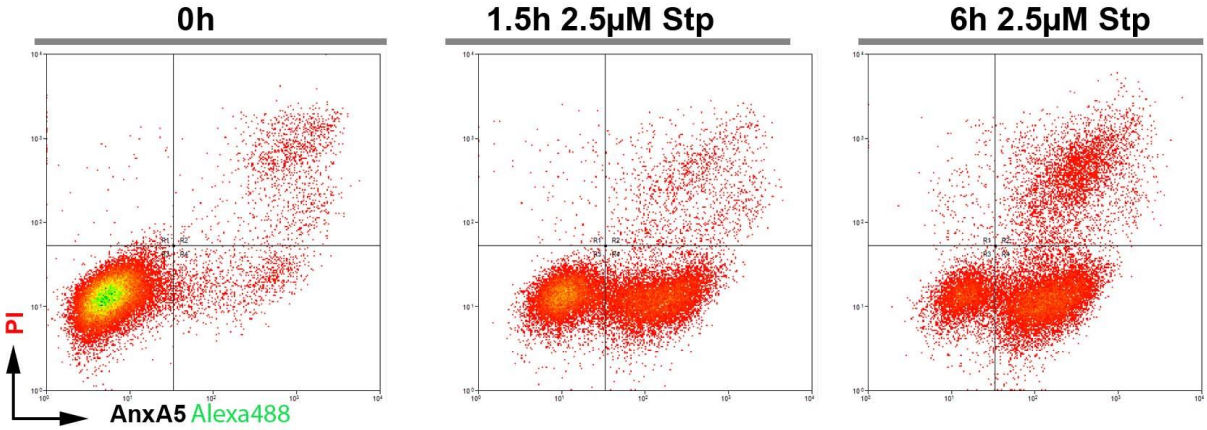

Fig. 1. Flow cytometry analysis of apoptosis. NIH 3T3 cells were cultured in the presence of 2.5 µM staurosporine (Stp) for 0, 1.5 or 6h to induce apoptosis. Subsequently, cells were harvested and stained with AnxA5-Alexa488 as well as propidium iodide. Annexin A5 binds to apoptotic (lower right quadrant) as well as to secondary necrotic cells (upper right quadrant).

References:

1. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C (1995). A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J Immunol Methods* **184**, 39-51.

2. Rosenbaum S, Kreft S, Etich J, Frie C, Stermann J, Grskovic I, Frey B, Mielenz D, Pöschl E, Gaipl U, Paulsson M, Brachvogel B (2011). Identification of Novel Binding Partners (Annexins) for the Cell Death Signal Phosphatidylserine and Definition of Their Recognition Motif. *Journal of Biological Chemistry* **286**(7): 5708-5716.

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