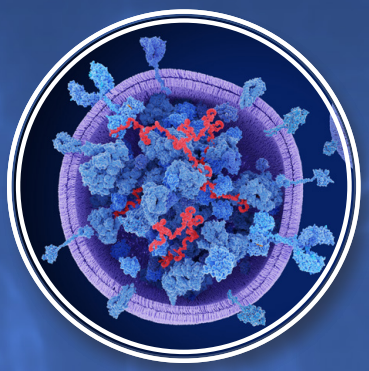
INNOVATIVE
EXTRACELLULAR
VESICLES FOR R&D

ALK-4 EVs
Data sheet
Ref. X141



EVs

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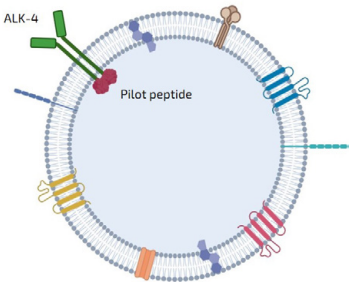
EVs bioengineered with human ALK-4



Schematic representation of ALK-4 EVs
ALK-4 is fused to the Pilot peptide in its C-terminal end, after removal of its cytoplasmic domain. Pilot peptide allows sorting to EVs.
Created using Biorender.com

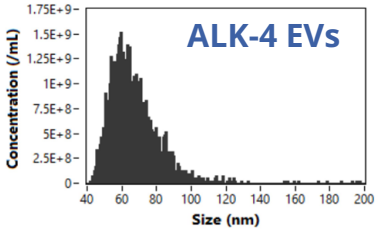
FORM	• EV suspension in PBS 1X - BSA 10 µg/mL • Produced by HEK293T cells • Sterile
AMOUNT	• 50 µg of pure EVs ; ≥ 2 x 10^{10} particles
APPLICATIONS	• Analysis of molecular interactions with ligands, specific antibodies or small molecules
STORAGE	• Store sterile at +4°C for up to 6 months

Small Extracellular Vesicles or EVs with a size of 30 - 150 nm diameter (including exosomes) are naturally released by most living cells. They are involved in cell physiological and pathological processes, transferring information from donor cells to recipient cells. They carry a variety of cargoes such as proteins, RNAs, lipids and DNA which can be captured by other cells.
Ciloa technology relies on active sorting of any kind of protein to the EVs produced by eukaryotic cells. A synthetic DNA optimised for coding human Activin receptor-like kinase 4 (ALK-4, UniProt ID: P36896), also named ACTR-IB (Activin receptor type-IB), a Transmembrane serine/threonine kinase activin type-1 receptor, is fused in C-terminus to a proprietary Pilot peptide (PP, for sorting to EVs) (Patent WO2009115561), after removal of its cytoplasmic domain. The resulting chimeric gene is cloned into Ciloa proprietary vector. This vector allows transcription in eukaryotic cells of a mRNA coding for the chimeric ALK-4-PP protein. Stably transfected human embryonic kidney (HEK) 293T cells lead to the expression of the target protein into EVs. The recombinant EVs produced are purified according to in-EV purification process, combining Tangential Flow Filtration and chromatography.
Pure ALK-4 EVs are subjected to standardised quality controls showing the total protein content, the number of particles, the EV size distribution, the presence of internal EV markers and the absence of cell protein contaminants. A direct ELISA on pure ALK-4 EVs reveals the CD81 surface marker.
Cellular expression of ALK-4 and its sorting with EVs, are given.
These ALK-4 EVs can be used to study the molecular interactions with ligands, specific antibodies or small molecules.

EV size distribution

The ALK-4 EV numbering and size distribution were obtained using nano-flow cytometry.

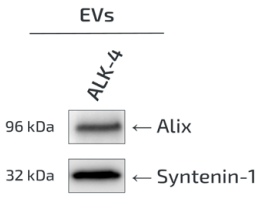
≥ 75 % of ALK-4 EVs have a size comprised between 50 and 80 nm, with a median size between 60 and 70 nm.



Detection of internal EV markers

Alix and Syntenin-1

The presence of Alix and Syntenin-1 was shown by Western blot analysis using EV-ID kit (in-EV, Ref. K211) with 5 µg of ALK-4 EVs as starting material.



Assessment of cellular contaminants

Calnexin

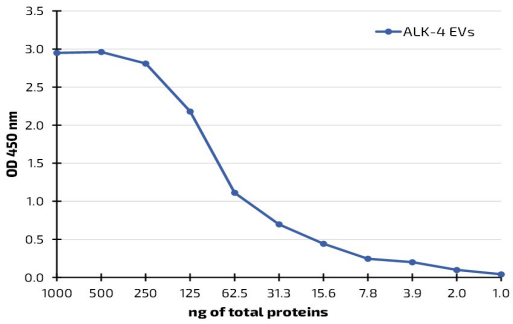
Undetectable. Calnexin amount quantified as < 0.63 ng/ml using a Human Calnexin ELISA Kit.

CD81 surface marker presence on EVs

ELISA detection and titration of CD81 EV specific marker present on EVs

A dilution series of ALK-4 EVs (starting from 1 µg to 1 ng) was coated directly on a 96-well plate. An antibody specific for human CD81 allowed the EV surface detection of CD81.

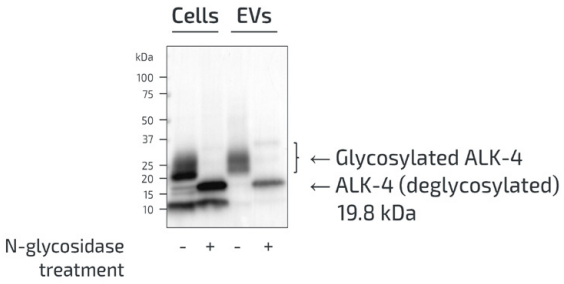
A result of this type is only achievable when EVs are sufficiently pure that their coating is not prevented by contaminating proteins.



ALK-4 cell expression and sorting with EVs

Cells extracts and EVs were subjected to Western blot analysis. ALK-4 fusion protein was detected using an anti-pilot peptide antibody.
ALK-4 acquires post-translational modifications such as N-glycosylation.

A band around 20-25 kDa, corresponding to ALK-4-PP, is observed in ALK-4 cell extract and EV samples. A thin band around 15-20 kDa, corresponding to ALK-4-PP (calculated MW: 19.8 kDa), is also observed in ALK-4 cell extract and EV samples after treatment with N-glycosidase.



> Glycosylated ALK-4 is sorted with EVs

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