Antibody Validation Report

aVR.APOA1.P02647.AB 1238779.v1.0 (January 4 2021)

HUBMAP

A. Basic Target Information

Target Information

UniProt Accession Number: P02647 **Target Name:** Apolipoprotein A-I

Antibody Information RRID: AB 1238779

Antibody Name: [A03] GOAT ANTI-HUMAN APOLIPOPROTEIN AI POLYCLONAL ANTI

Host Organism: Goat Clonality: Polyclonal

Vendor: Academy Bio-Medical Company, Inc.

Catalog Number: 11A-G2 Lot Number: 012075 Recombinant (Y/N): No

Organ/Tissue used for validation: Serum

HuBMAP Platform Used: IP-MS

Protocols.io doi for Validation Protocol: 10.17504/protocols.io.brp9m5r6

ORCID ID of submitter: 0000-0002-4549-2585

B. Validation Data

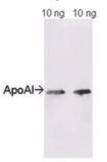
B.1. Vendor Validation: IP, WB

Date Accessed: 01/07/21

URL: https://www.academybiomed.com/products/a03-goat-anti-human-apolipoprotein-ai-polyclonal-antibody

Western blotting

Western Blot Data for Affinity Purified Goat Anti-Human ApoAl Antibody

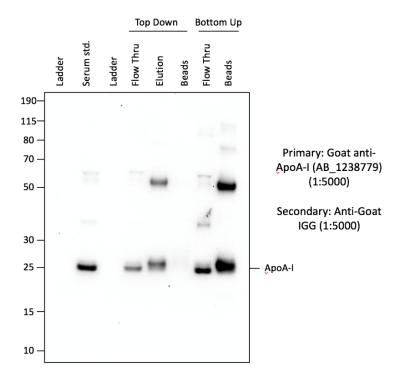


- 1. Human ApoAl (ng)
- Goat anti-Human ApoAl (1:1000)
- HRP Dunkey-anti-Goat IgG (1:5000)
- 4. 12 % Gel

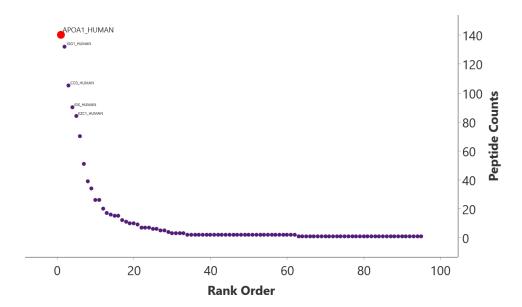
B.2. Laboratory Validation:

Immunoprecipitation/Western Blot

Western Blot of ApoA-I Immunoprecipitation from Commercial Serum



Immunoprecipitation/Bottom-Up MS



Immunoprecipitation/Top-Down MS

Proteoforms Identified:

1: PFR00000053599, ApoA1 (25-267), 28,062.482

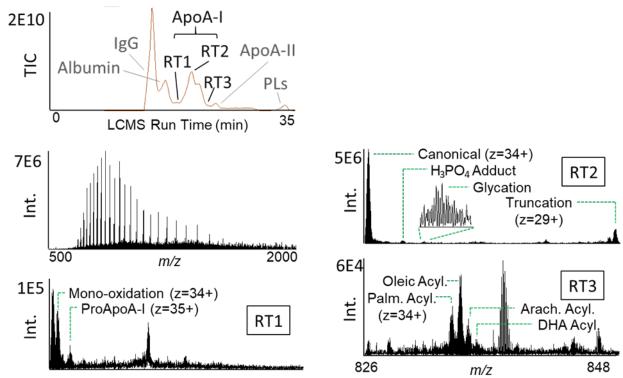
DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQE FWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQE KLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPA LEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ

2: PFR00000056138, ProApoA1 (19-267), 28,944.906,

RHFWQQDEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQ LGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGARQ KLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLSTL SEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ

- 3: PFR00000073908, Truncated ApoA1 (25-266), 27,934.423,
- DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQE FWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQE KLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPA LEDLRQGLLPVLESFKVSFLSALEEYTKKLNT
- 4: PFR00000216392, ApoA1 (25-267) Mono-oxidation, 28,078.476, DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQE FWDNLEKETEGLRQEM[MOD:00425]SKDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGA RQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLS TLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ
- 5: PFR00005052085, ApoA1 (25-267) Mono-oxidation, 28,078.476, DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQE FWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEM[MOD:00425]ELYRQKVEPLRAELQEGA RQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLS TLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ
- 6: PFR00000216398, ApoA1 (25-267) Palmitoylated, 28,299.70, DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQE FWDNLEKETEGLRQEMSK[MOD:00440]DLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGA RQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLS TLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ
- 7: PFR00000216399, ApoA1 (25-267) Oleoylated, 28,325.72 DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQE FWDNLEKETEGLRQEMSK[MOD:02012]DLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGA RQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATEHLS TLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ





LC-TDMS results on Fusion Lumos. Different peaks correspond to elution times of different proteins and molecules (e.g. immunoglobulin G, IgG; phospholipids, PL). Proteoforms of ApoA-I elute at three different retention times. At the middle, the Full MS scan at the main retention time of ApoA-I proteoforms (RT2). At the bottom, SIM scans set between two charge states of canonical ApoA-I, at the different retention times. Notably, a different profile of proteoforms of ApoA-I is observable at the different retention times. At RT3, ApoA-I modified by different fatty acids (from left to right: palmitic, oleic, arachidonic and docohexaenoic acylations), can be observed.

Protter (Omasits et al., Bioinformatics. 2013 Nov 21)

