ProductInformation

APOLIPOPROTEIN E3
Human, Recombinant

Product Code **A2331**
Storage Temperature –20°C

Synonyms: Human rApoE3

Product Description
This product is human recombinant apolipoprotein E3 produced by Baculovirus-mediated expression in Spodoptera frugiperda cells.[1]

Apolipoprotein E (ApoE) has long been known to be a ligand for the low density lipoprotein (LDL) receptor, and has recently been implicated as an important factor in nerve degeneration and regeneration. Ongoing research points to new roles for ApoE in neuron function, and suggests potential ways in which the protein may be involved in the development of new therapeutics for crippling diseases in the neurological sciences.

The discovery by Corder et al.[2] that apolipoprotein E (ApoE) isoforms are associated with the progression of Alzheimer's disease in late-onset families renewed the interest in the function of this important member of the apolipoprotein family. Researchers have shown that there is a prevalence of Alzheimer's disease in individuals with the ApoE4 isoform. ApoE's role in Alzheimer's disease is unclear. In one hypothesis, ApoE4 is less potent than ApoE3 in inhibiting nucleation of amyloid formation.[3] Another theory focused on the ability of ApoE3 and E2 to stabilize the neuronal microtubule protein tau, preventing nerve cell death. Sigma's recombinant human ApoE2, E3 and E4 isoforms retain full biological activity, enabling researchers to study interactions of ApoE isoforms with beta-amyloid and tau proteins as well as the LDL receptor.

The molecular weight of rApoE is approximately 34,000 as determined by SDS-PAGE. However, under non-denaturing and nonreducing conditions, Apo E can form dimers and multimers because it is an amphipathic protein. This aggregation results in ApoE remaining in the well. The presence of urea or guanidine-HCl in the gel can prevent this. Two-dimensional electrophoresis performed according to the method of O'Farrell indicates a pI for the primary isoform of approximately 6.25 for rApoE2, 6.35 for rApoE3, and approximately 6.55 to 6.7 for rApoE4. Minor acidic isoforms are also present and appear to be due to glycosylation and deamination differences.
The DNA clones used for the expression of recombinant human ApoE2, E3, and E4 were derived from clones originally isolated in the 1980s by prominent ApoE researchers, primarily at the J. David Gladstone Institutes. The pI values of ApoE2, E3, and E4 differ because of the unique amino acid substitutions described above. The amino acid sequence of ApoE2, E3, and E4 differ in the following way:

	Residue	
	112	158
E2	Cys	Cys
E3	Cys	Arg
E4	Arg	Arg

Two-dimensional gel electrophoresis reveals a complicated isoform pattern for hrApoE, reminiscent of the human serum ApoE pattern.[4] Along with the primary hrApoE band, three to four additional bands occur at more acidic isoelectric points (pI), apparently representing sialated and deaminated forms of hrApoE. The relative abundance of each recombinant isoform is in the range seen with human serum ApoE.

Vial content: 100 µl of solution containing 50 µg protein (0.50 mg/ml) in 0.7 M ammonium bicarbonate

Protein is determined by Bradford method with BSA as standard. Because ammonium bicarbonate interferes with the assay, this buffer should be included in all standards and blanks. Do not use other common protein assays such as the Lowry and BCA assays.

Preparation Instructions
Human rApoE is soluble in aqueous solutions. At concentrations greater than 1.0 mg/ml there may be no free monomer due to self-association common to the amphipathic apoproteins.[5]

Storage/Stability
ApoE may aggregate with repeated freeze-thaws. Freezing ApoE in ammonium bicarbonate solutions is the preferred method of storage. Avoid storage at 4°C.[6] In order to remove the ammonium bicarbonate, the solution should be dialyzed into the desired buffer. Lyophilization of ApoE is not recommended because

some biological activities may be affected. In order to minimize self-association and aggregation of lyophilized samples, dissolve lyophilized powders in 0.7 M ammonium bicarbonate. Aggregates and multimers may still exist.

Specificity

Human recombinant ApoE3 and ApoE4 compete with iodinated human low density lipoprotein for binding to the human Apo B/E (LDL) receptor 1. ApoE2 cannot compete for binding to the LDL receptor due to its amino acid substitution at position 158.[1] Human recombinant ApoE also binds to amyloid peptide in a soluble binding assays.[7]

References

1. Gretch, D., et al. Proc. Natl. Acad. Sci. USA, **8,** 8530 (1991).
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4. Zannis, V.I., and Breslow, J.L., Mol. Cell. Biochem., **42,** 3-20 (1982).
5. Formisano, S., et al., J. Biol. Chem., **253,** 354 (1978).
6. Rall, S.C., et al., Methods Enzymol., **128,** 273 (1986).
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