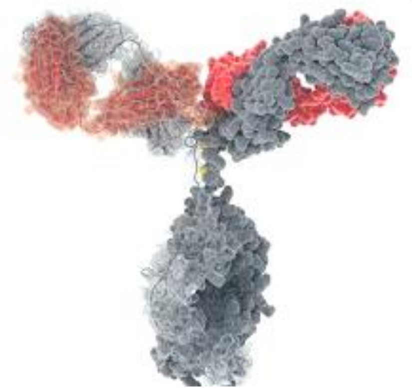
NADINE Meeting in Brussel
September 22nd, 2015
Partner MB



WP5:

- Task 5.2: Antibody development, production and characterisation

Collaborations with partners in NADINE (WP1, WP3)

- UULM (P06), UKES (P07), UPCE (P03), MACRO (P04), DS (P08), ICN (P14) and UPS (P02)

Development of Aβ–peptide specific antibodies

Commercially available antibodies with known epitope:

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
(1-42)

6E10 — FRHDS; 1E8 — VFFAE; 12F14 — GGVVIA

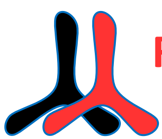
Newly developed monoclonal antibodies available in large quantities:

AEF1.1-AEF1.19 — KLV1.1-KLV3.1 — No success in development of MAb

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
(1-42)

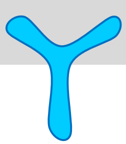
Newly developed polyclonal antibodies available in large quantities:

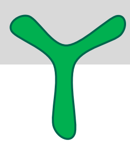
Rabbit sera 54, 76



Rabbit sera 77, 128 and 24

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
(1-42)





Rabbit sera 17 — Rabbit sera 18

Development of rabbit sera to Aβ–peptide

The best rabbit polyclonal sera without special purification is 77

Rabbit polyclonal sera 77, 128 and 24

Rabbit sera 17

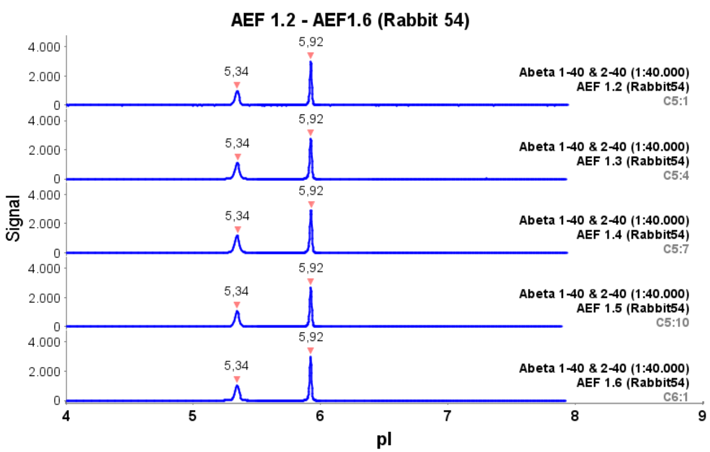
Rabbit sera 18

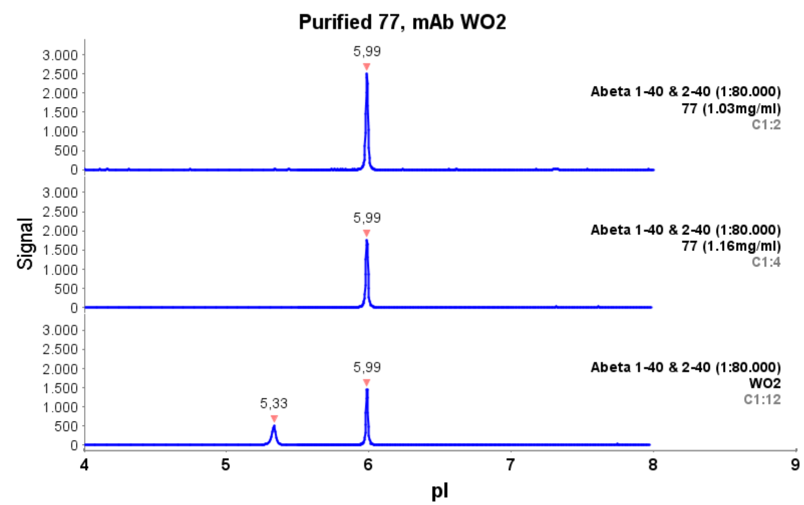
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (1-42)

1E8

12F14

Rabbit polyclonal sera 54, 76





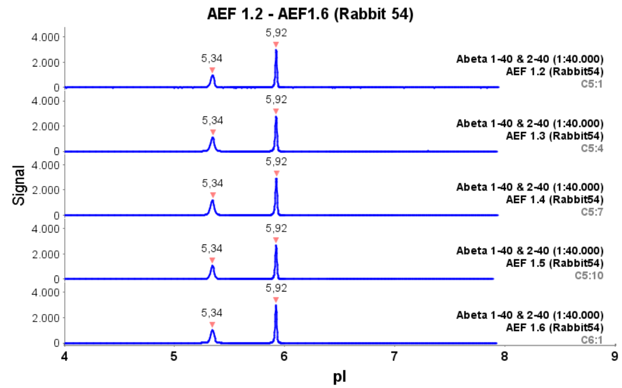
AEFRHDSGYEVHH (2-X)

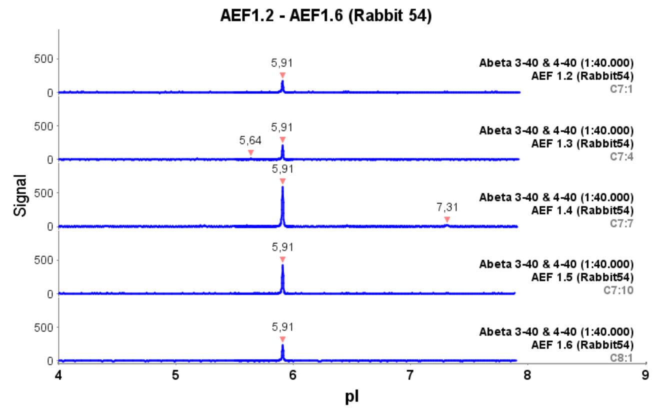
DAEFRHDSGYEVHH (1-X)

AEF1.1-AEF1.19

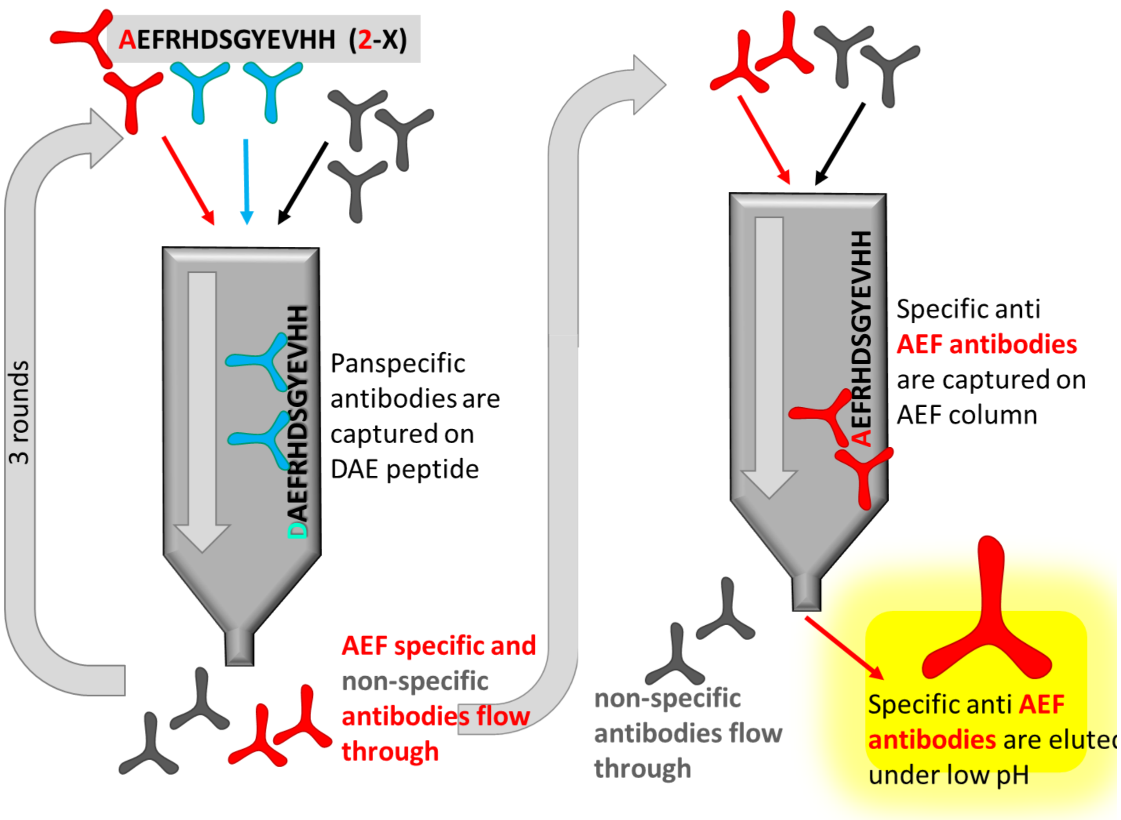
Rabbit polyclonal sera 54, 76, 77, 128 and 24

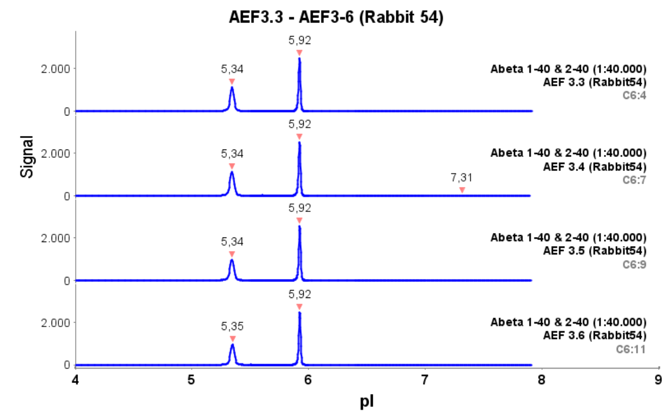
Newly developed method of purification rabbit sera (77, 76, 128 and 24)

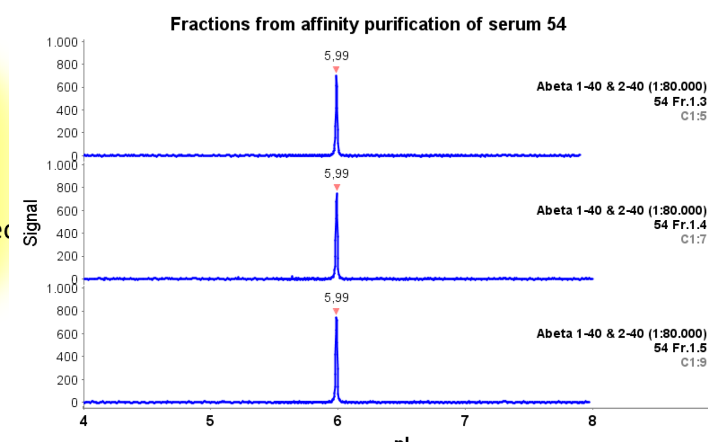




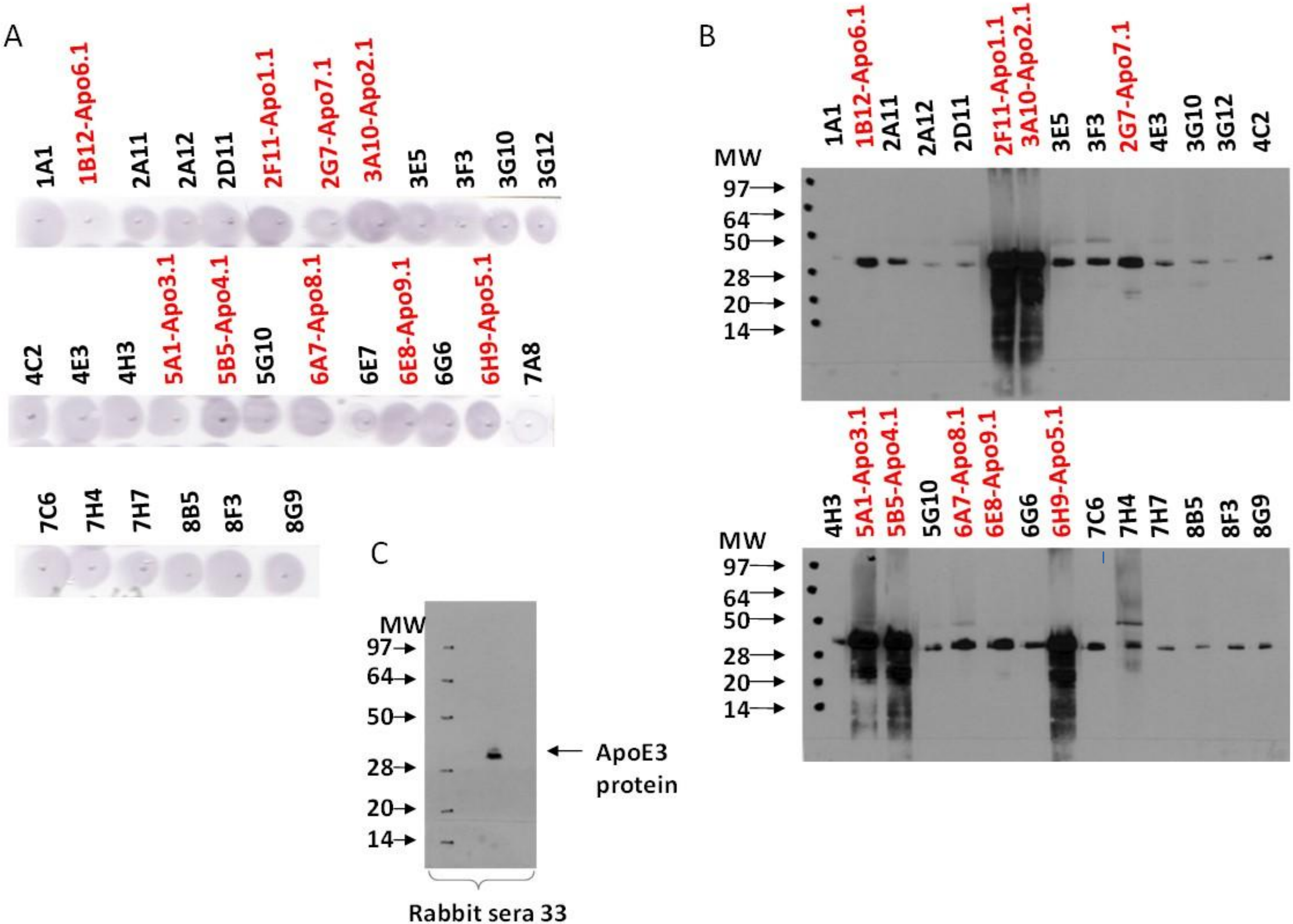
Rabbit polyclonal sera generated anti AEF:





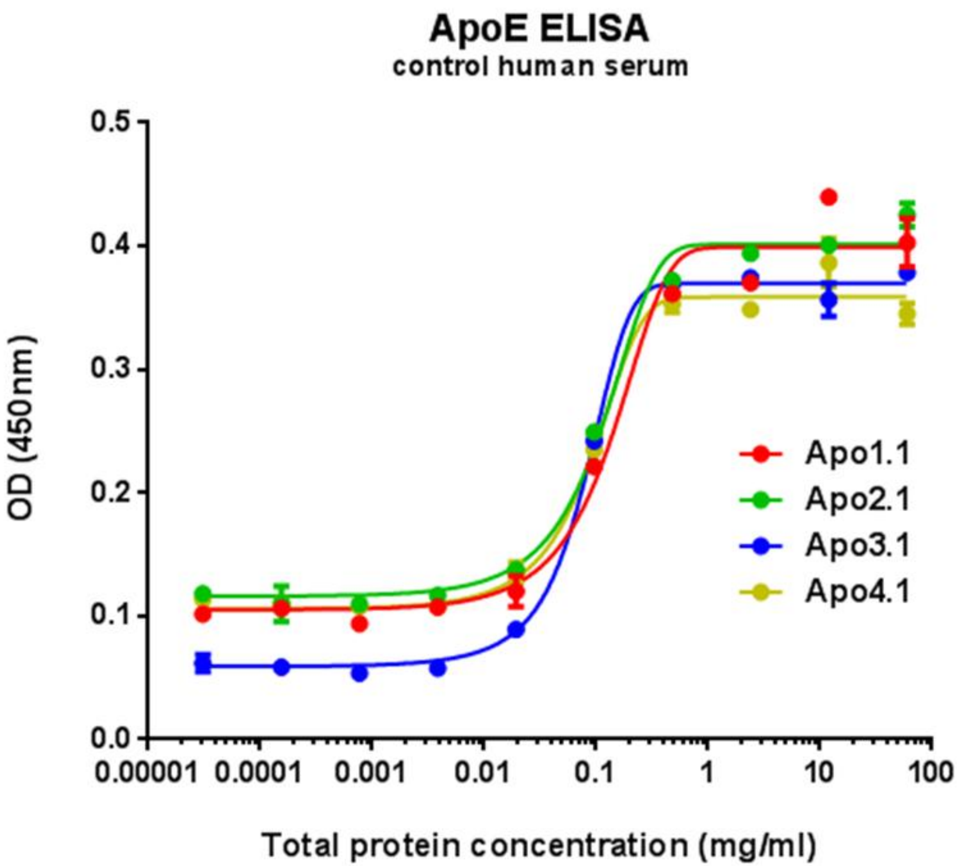


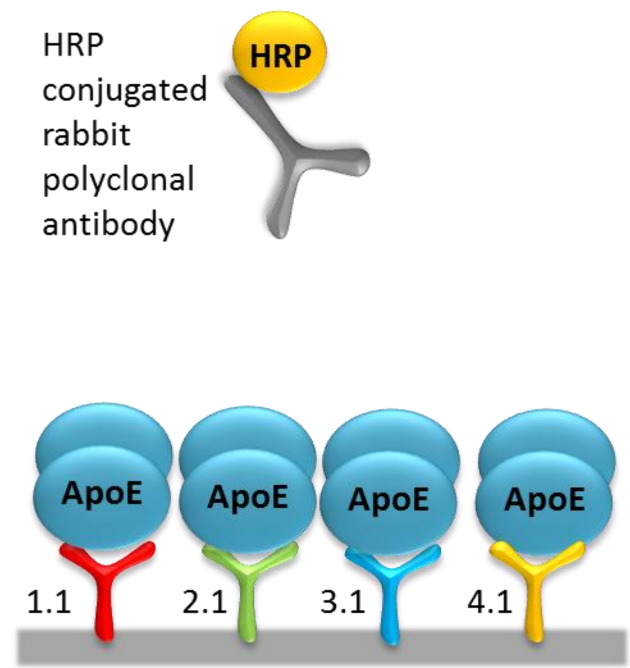
Summary of antibody development



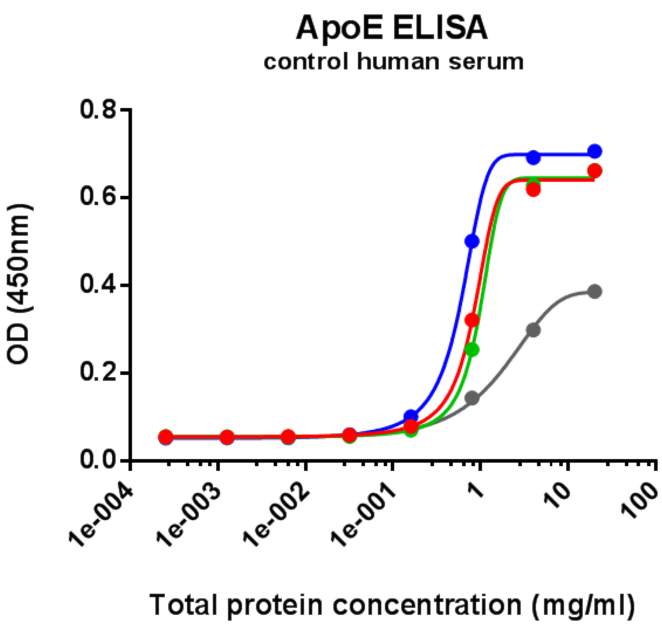
Development of antibodies to ApoE plus ELISA using combination of mouse monoclonal antibody and rabbit sera

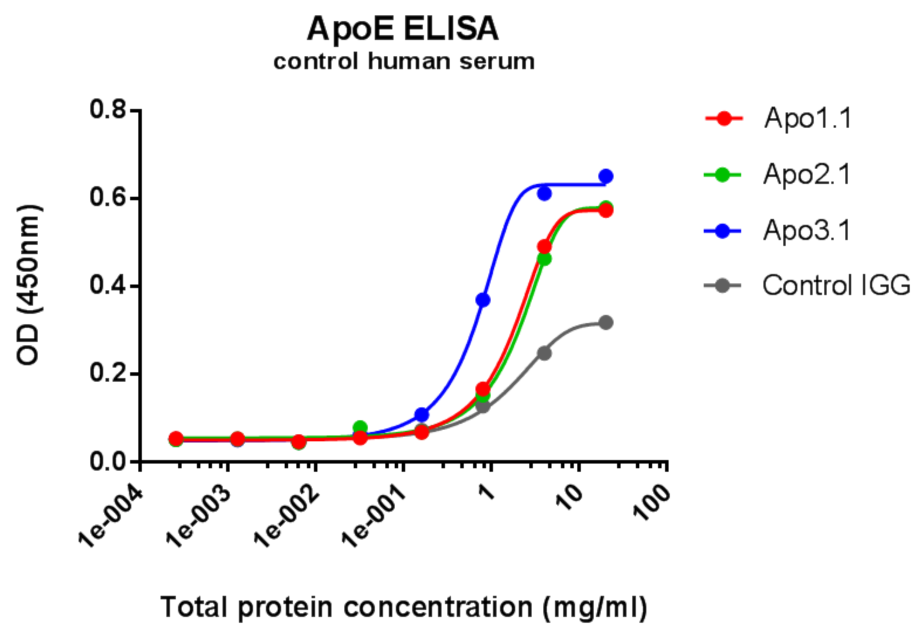
Rabbit polyclonal sera developed: number 33
Mouse monoclonal antibody developed: ApoE1.1 – ApoE5.1

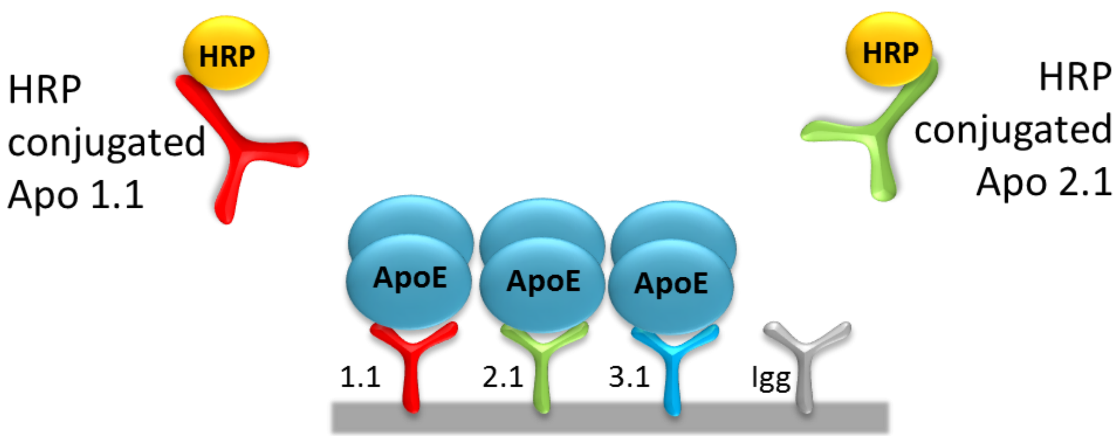




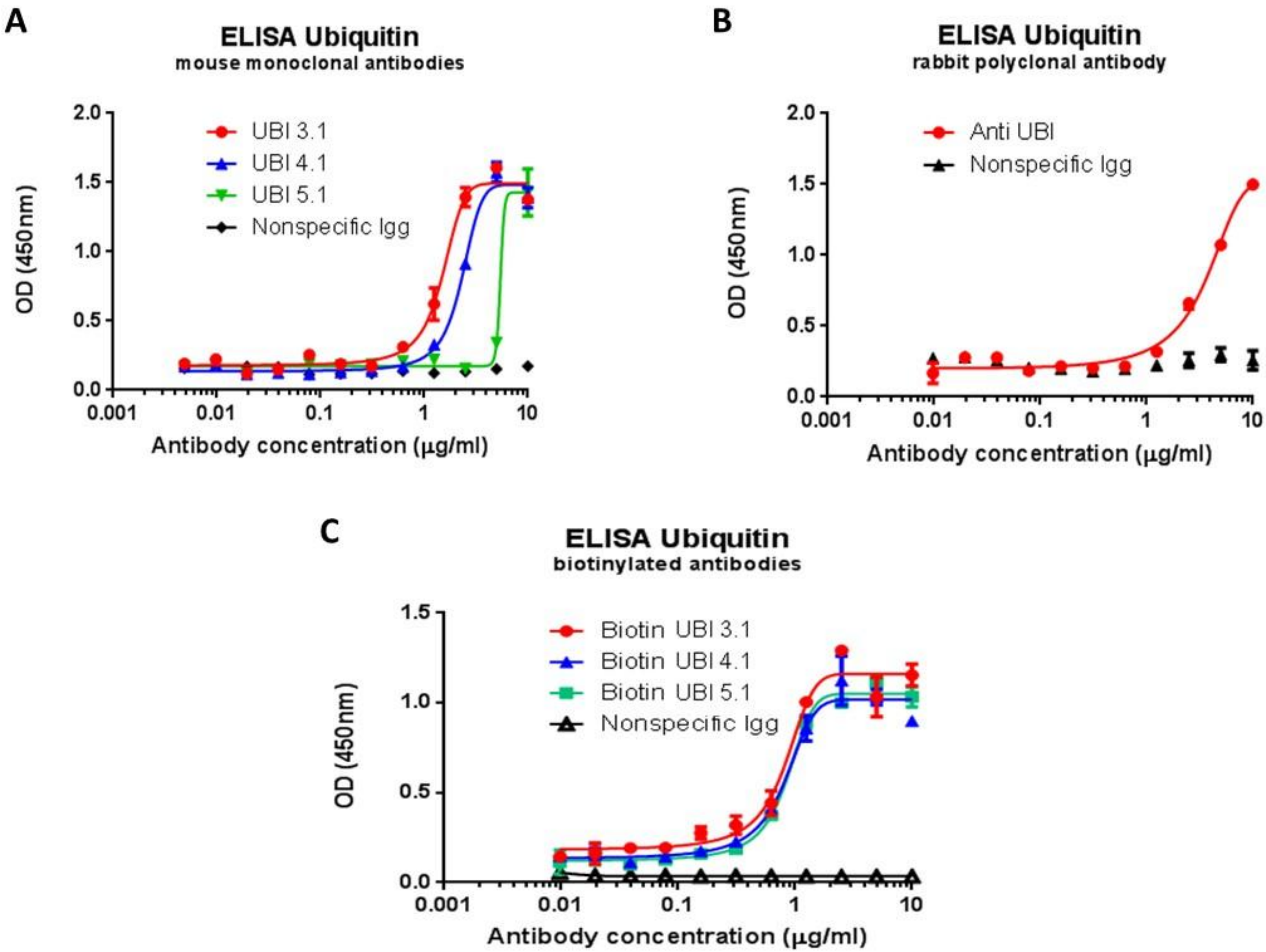
Development of ApoE ELISA using combination of two mouse monoclonal antibodies



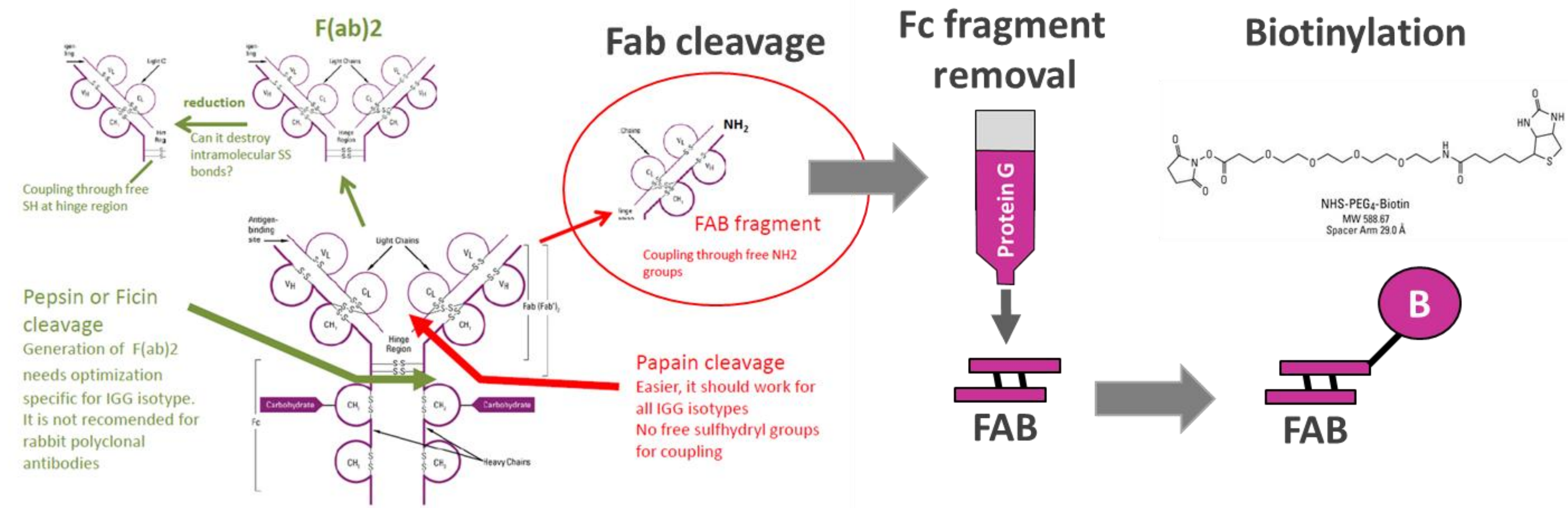


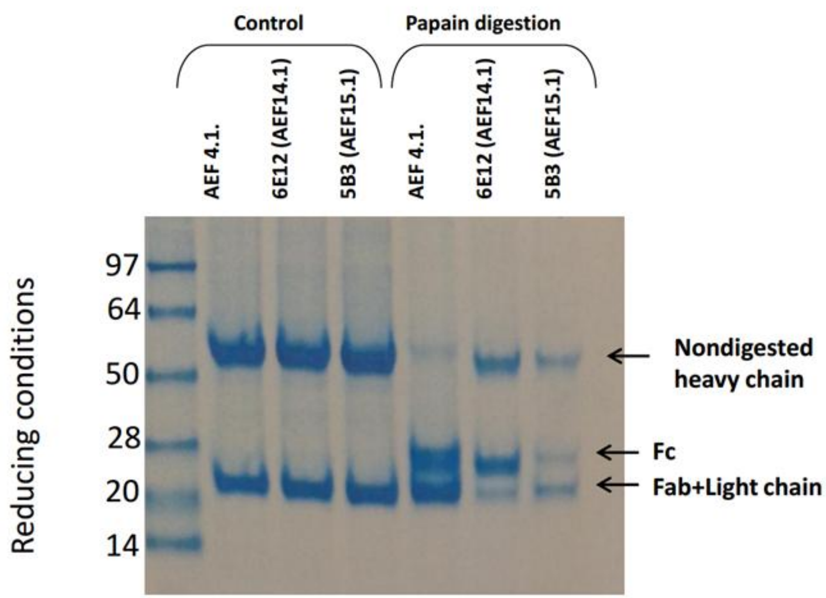


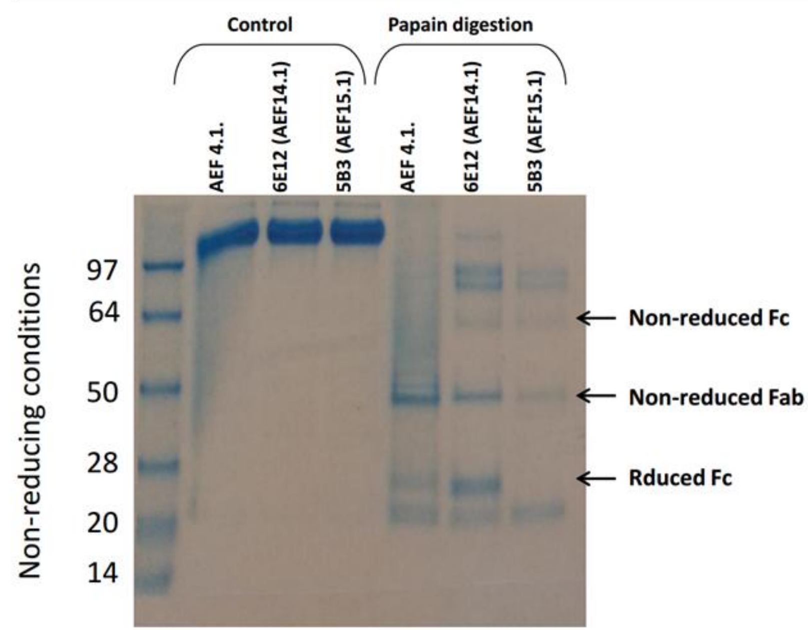
Summary of antibody to ubiquitine development



Preparation of biotinylated FAB fragments







Summary of antibody development

Antibodies anti N-terminal Ab-peptide

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
A-beta peptide: AEFRHDSGYEVHHC	**AEF2.1**	Mouse monoclonal antibody clone AEF2.1	Not required but available for direct labelling	IgG2b, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF4.1**	Mouse monoclonal antibody clone AEF4.1	Not required but available for direct labelling	IgG2b, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF8.1**	Mouse monoclonal antibody clone AEF8.1	Not required but available for direct labelling	IgG2b, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF11.1**	Mouse monoclonal antibody clone AEF11.1	Not required but available for direct labelling	IgG2b, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF12.1**	Mouse monoclonal antibody clone AEF12.1	Not required but available for direct labelling	IgG2b, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF14.1**	Mouse monoclonal antibody clone AEF14.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF15.1**	Mouse monoclonal antibody clone AEF15.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF16.1**	Mouse monoclonal antibody clone AEF16.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF17.1**	Mouse monoclonal antibody clone AEF17.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
A-beta peptide: AEFRHDSGYEVHHC	**AEF18.1**	Mouse monoclonal antibody clone AEF18.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**

Summary of antibody development

Antibodies anti Ab-peptide (middle part)

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
A-beta peptides mixture: CEVHHQKLVFFAED and CKLVFFAEDVGSNK	**KLV1.1**	Mouse monoclonal antibody clone KLV1.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
A-beta peptides mixture: CEVHHQKLVFFAED and CKLVFFAEDVGSNK	**KLV2.1**	Mouse monoclonal antibody clone KLV1.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
A-beta peptides mixture: CEVHHQKLVFFAED and CKLVFFAEDVGSNK	**KLV3.1**	Mouse monoclonal antibody clone KLV1.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**

Summary of antibody development

Rabbit polyclonal antibodies anti Ab-peptide

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
A-beta peptide: N-terminal part DAEFRHDSGYEC	**Rabbit sera 76**	Affinity purified rabbit sera 76	None	IgG	**Characterised and available**
A-beta peptide: N-terminal part DAEFRHDSGYEC	**Rabbit sera 76-biotin**	Affinity purified rabbit sera 76	Labelled with biotin	IgG	**Characterised and available**
A-beta peptide: N-terminal part DAEFRHDSGYEC	**Rabbit sera 54**	Affinity purified rabbit sera 54	Not required but available for direct labelling	IgG	**Characterised and available**
A-beta peptide: N-terminal part AEFRHDSGYEVHHC	**Rabbit sera 77**	Affinity purified rabbit sera 77	Not required but available for direct labelling	IgG	**Characterised and available**
A-beta peptide: N-terminal part AEFRHDSGYEVHHC	**Rabbit sera 128**	Affinity purified rabbit sera 128	Not required but available for direct labelling	IgG	**Ready for partners upon requirement**
A-beta peptides mixture: CEVHHQKLVFFAED and CKLVFFAEDVGSNK	**Rabbit sera 17**	Affinity purified rabbit sera 17	Not required but available for direct labelling	IgG	**Ready for partners upon requirement**
A-beta peptide: CNKGAIIGLMVGG	**Rabbit sera 18**	Affinity purified rabbit sera 18	Not required but available for direct labelling	IgG	**Ready for partners upon requirement**

Summary of antibody development

Available antibodies to monoubiquitine

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
Ubiquitin	**Rabbit sera 37**	Affinity purified rabbit sera 37	None	IgG	**Ready for partners upon requirement**
Ubiquitin	**Rabbit sera 37-biotin**	Affinity purified rabbit sera 37	Labelled with biotin	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI3.1**	Mouse monoclonal antibody clone UBI3.1	None	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI3.1-biotin**	Mouse monoclonal antibody clone UBI3.1	Labelled with biotin	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI3.1-HRP**	Mouse monoclonal antibody clone UBI3.1	Labelled with horse radish peroxidase	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI3.1-AP**	Mouse monoclonal antibody clone UBI3.1	Test labelling with Alkaline phosphatase	IgG1 kappa	**Ready for additional labelling upon requirement**
Ubiquitin	**UBI4.1**	Mouse monoclonal antibody clone UBI4.1	None	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI4.1-biotin**	Mouse monoclonal antibody clone UBI4.1	Labelled with biotin	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI4.1-HRP**	Mouse monoclonal antibody clone UBI4.1	Labelled with horse radish peroxidase	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI4.1-AP**	Mouse monoclonal antibody clone UBI4.1	Test labelling with Alkaline phosphatase	IgG1 kappa	**Ready for additional labelling upon requirement**
Ubiquitin	**UBI5.1**	Mouse monoclonal antibody clone UBI5.1	None	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI5.1-biotin**	Mouse monoclonal antibody clone UBI5.1	Labelled with biotin	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI5.1-HRP**	Mouse monoclonal antibody clone UBI5.1	Labelled with horse radish peroxidase	IgG1 kappa	**Ready for partners upon requirement**
Ubiquitin	**UBI5.1-AP**	Mouse monoclonal antibody clone UBI5.1	Test labelling with Alkaline phosphatase	IgG1 kappa	**Ready for additional labelling upon requirement**

Summary of antibody development

Available antibodies to polyubiquitine

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
Polyubiquitine peptide CTLEVEPSDTIENVK	Rabbit sera 16	Affinity purified rabbit sera 16	None	IgG kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI2.1	Mouse monoclonal antibody clone P-UBI2.1	None	IgG1 kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI3.1	Mouse monoclonal antibody clone P-UBI3.1	None	IgG1 kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI4.1	Mouse monoclonal antibody clone P-UBI4.1	None	IgG1 kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI6.1	Mouse monoclonal antibody clone P-UBI6.1	None	IgG1 kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI7.1	Mouse monoclonal antibody clone P-UBI7.1	None	IgG1 kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI8.1	Mouse monoclonal antibody clone P-UBI8.1	None	IgG1 kappa	Ready for partners upon requirement
Polyubiquitine peptide CTLEVEPSDTIENVK	P-UBI9.1	Mouse monoclonal antibody clone P-UBI9.1	None	IgG1 kappa	Ready for partners upon requirement

Summary of antibody development

Available antibodies to ERK1/2

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
ERK1/2: peptide KTGFLT(p)EY(p)VATR	**Rabbit sera 78**	Affinity purified rabbit sera 78	None	IgG	**Characterised and available**
ERK1/2: peptide KTGFLT(p)EYVATR	**Rabbit sera 59**	Affinity purified rabbit sera 59	None	IgG	**Characterised and available**
ERK1/2: peptide KTGFLTEY(p)VATR	**Rabbit sera 56**	Affinity purified rabbit sera 56	None	IgG	**Characterised and available**
ERK1/2: protein	**Rabbit sera 32**	Rabbit polyclonal antibody 34 (sera)	None	Whole sera	**Available for additional testing**
ERK1/2: protein	**ERK1.1**	Mouse monoclonal antibody clone ERK1.1	None	Ig**G1**	**Available for additional testing**

Summary of antibody development

Available antibodies to TDP43 and ApoE3

Antigen	Name of antibody	Type of antibody	Labelling with required label	Isotype	Status
Human TDP43 protein	**Rabbit sera 31**	Rabbit polyclonal antibody 31 (sera)	None	Whole sera	**Available for additional testing**
Human ApoE3 protein	**Rabiit sera 33**	Rabbit polyclonal antibody 33 (sera)	None	Whole sera	**Ready for partners upon requirement**
Human ApoE3 protein	**Apo1.1**	Mouse monoclonal antibodies Apo1.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
Human ApoE3 protein	**Apo2.1**	Mouse monoclonal antibodies Apo2.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
Human ApoE3 protein	**Apo3.1**	Mouse monoclonal antibodies Apo3.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
Human ApoE3 protein	**Apo4.1**	Mouse monoclonal antibodies Apo4.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**
Human ApoE3 protein	**Apo5.1**	Mouse monoclonal antibodies Apo5.1	Not required but available for direct labelling	IgG1, kappa	**Ready for partners upon requirement**