

Final Report

Size-Exclusion Chromatography Analysis

Report number:	PA-R34480B-1
Report date:	27 th November 2025
Project number:	34480
Client name:	Not Applicable
Client organisation:	APAF
Client address:	Level 4, 4 Wally's Walk, Macquarie Park, New South Wales, 2113
Client contact number:	(02) 9850 6216
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Date sample(s) received:	18 th November 2025
Number of samples:	One (2)
Project leader:	Prashina Singh
Authorised by:	Dr. David Cantor
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Attachments	No

The results apply to the sample(s) as received.

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As per [APAF Terms and Conditions](#), samples will be retained for a period of thirty (30) days and testing records will be accessible for a period of three (3) years from the date of reporting results unless other arrangements have been made; refer to Clause 11.1 (sample retention) and Clause 10.3 (test records) for conditions that apply.

Acknowledgment: To comply with our NCRIS (National Research Infrastructure for Australia) operating grant, we request that any publication arising from access to the facility acknowledge the contribution of APAF staff and include the statement "*This study/project/research used NCRIS-enabled Australian Proteome Analysis Facility (APAF) infrastructure*".



Accreditation Number: 20344

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SAMPLE DETAILS

- Two samples were received for Size Exclusion Chromatography (SEC) analysis.
- Samples were received in good condition and were stored at room temperature prior to analysis.
- Sample identifications can be found in the results.

METHOD DETAILS

Size-exclusion Chromatography (SEC)

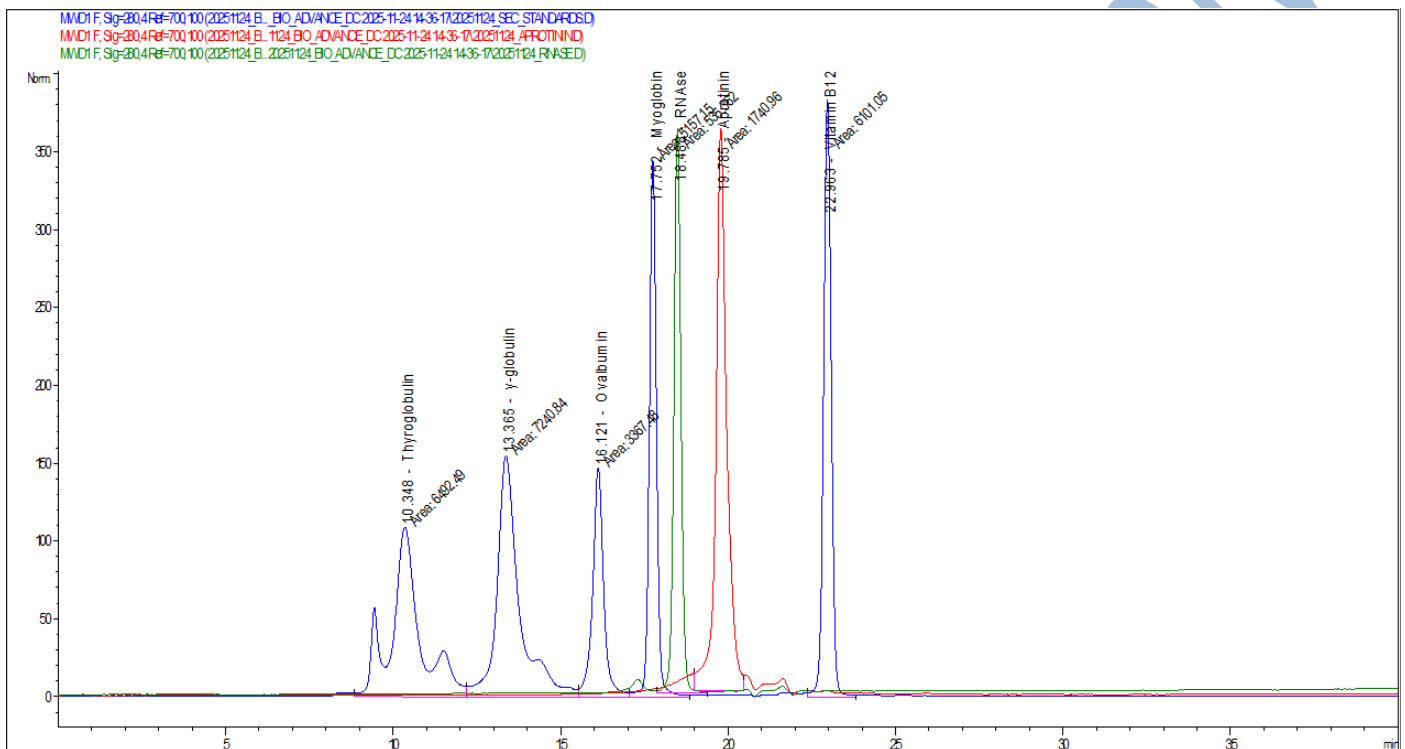
- Analysis was performed on the 24th of November 2025, upon an Agilent 1260 HPLC system, in accordance with APAF SOP LC-004 (Size Exclusion Chromatography (SEC) of Proteins).
- Samples were prepared as 2 mg/mL suspensions in SEC running buffer and filtered through a 0.22 µm syringe filter prior to analysis.
- A running buffer blank was also prepared.
- A purchased protein standard (Biorad Cat. # 1511901) was run to ensure resolution of the molecular weight range.
- Size exclusion chromatography was performed using an Agilent AdvanceBio SEC column with dimensions 7.8 x 300 mm, 2.7 µm.
- The chromatography conditions were:
 - Flow rate: 0.5 mL/min
 - Stop time: 40 min
 - Column temperature: 35 °C.
 - UV detection: 214 nm, 280 nm
 - Buffer/mobile phase: 100 mM sodium phosphate, pH 7, 250 mM NaCl.
- Injection volume: 10 µL.

RESULTS

Size-exclusion Chromatography (SEC)

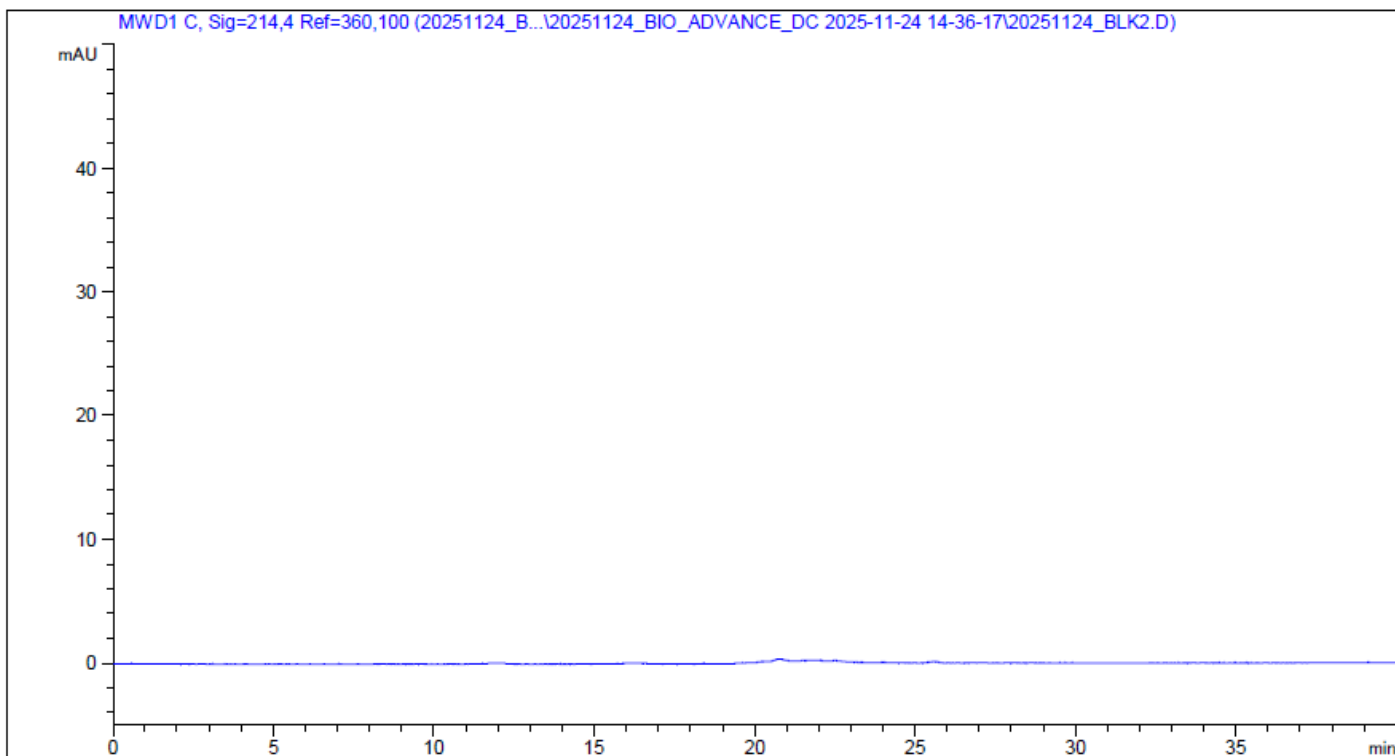
- Protein standards were run prior to sample analysis and showed the chromatographic conditions employed separated proteins over the molecular weight range of approx. 670 kDa - 1.3 kDa.
- Find on the following pages the chromatogram for protein standards, chromatogram and percentage area report for each sample.

SEC protein standards



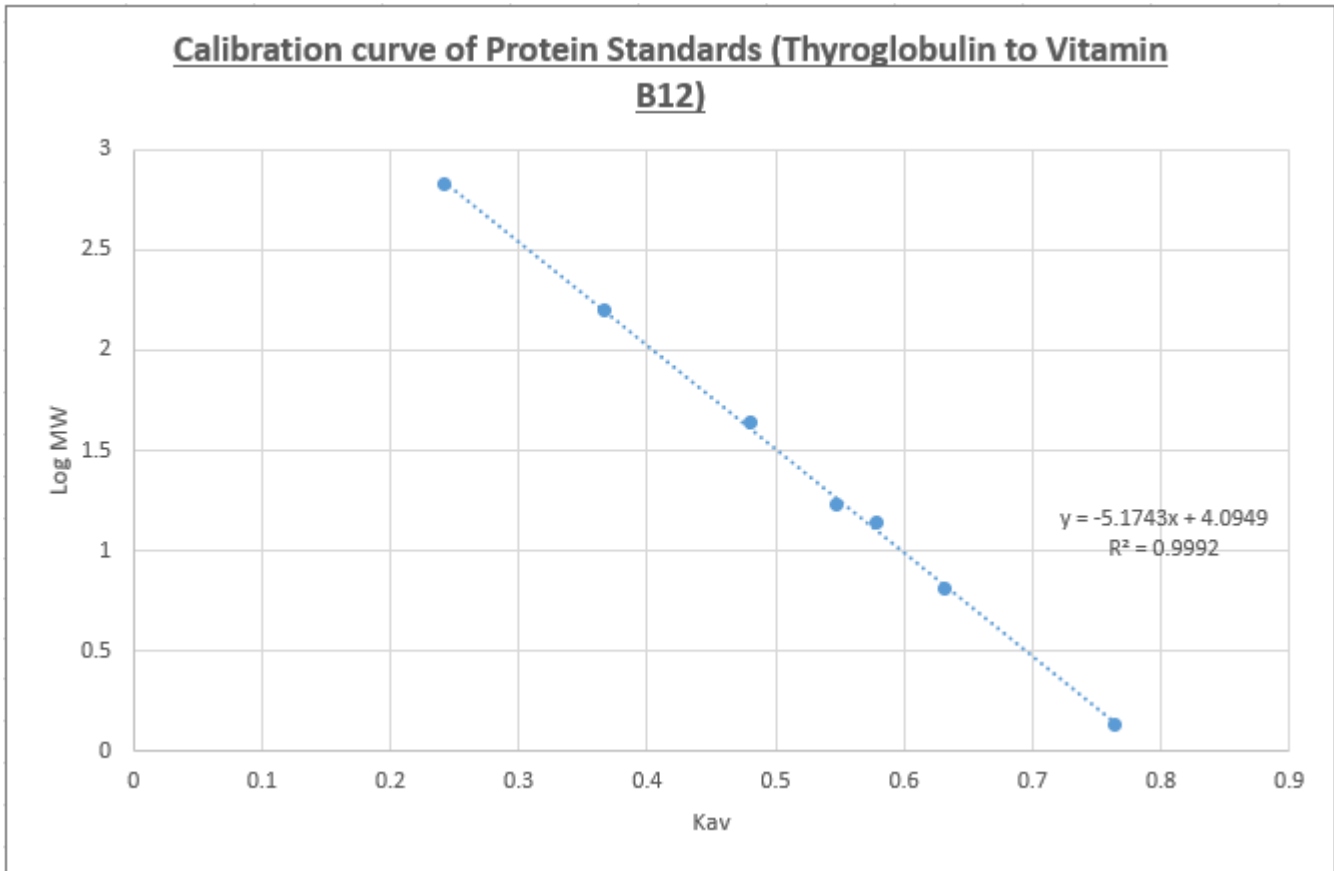
Name	MW (kDa)	Retention time (min)
Thyroglobulin	670	10.348
γ-globulin	158	13.365
Ovalbumin	44	16.121
Myoglobin	17	17.752
RNase	13.7	18.488
Aprotinin	6.5	19.785
Vitamin B12	1.35	24.291

SEC Buffer Blank



Example Report

Calibration curve



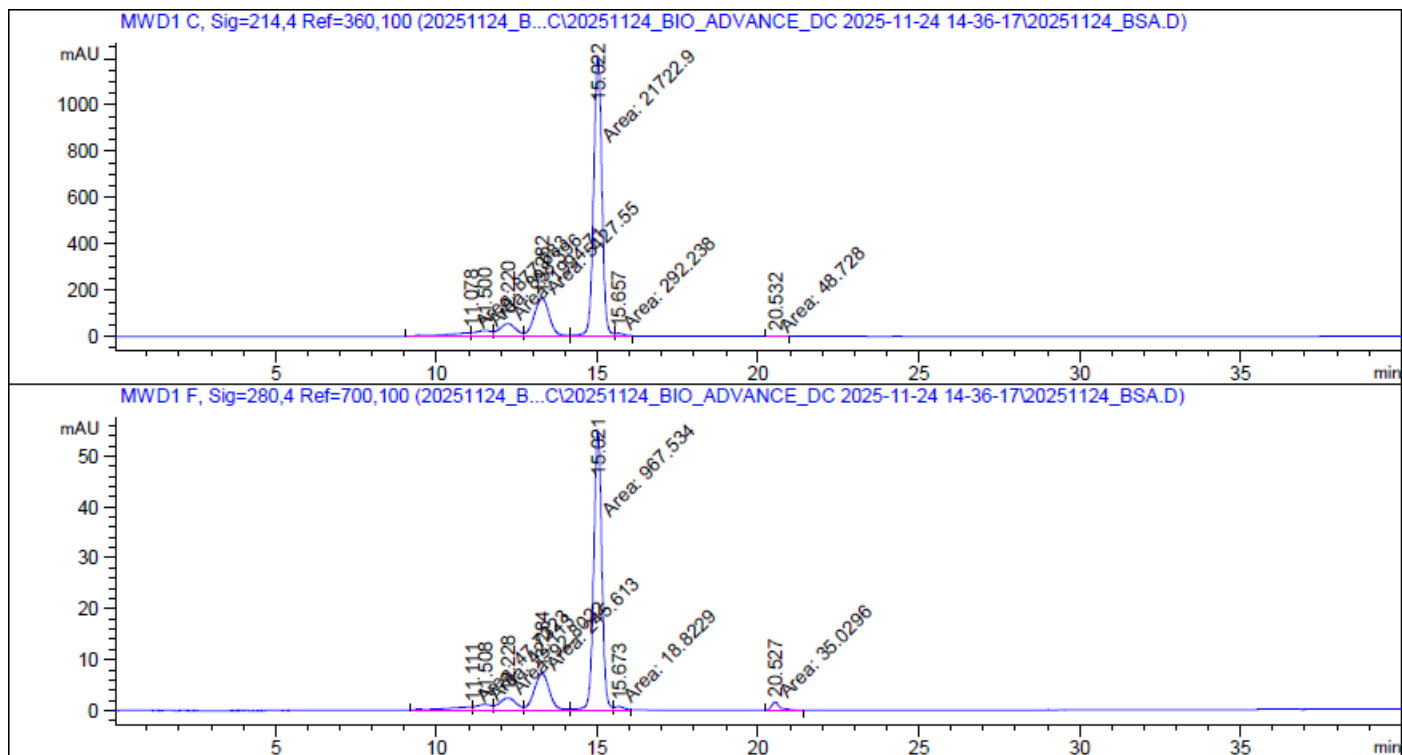
The following retention time windows were calculated from the calibration curve:

MW (kDa)	Retention time (min)
500	11.02
250	12.43
125	13.84
75	14.87
50	15.70
25	17.10
10	18.96
5	20.37
2.5	21.78
1	23.64

These retention times were used to determine the % area for the ranges > 500 kDa, 500-250 kDa, 250-125 kDa, 125-75 kDa, 75-50 kDa, 50-25 kDa, 25-10 kDa, 10 – 5 kDa, 5 - 2.5 kDa, 2.5 - 1 kDa, < 1kDa + retained peptides.

Bovine Serum Albumin Test Sample 1 (APAF Sample ID – n/a)

Peak form – based integration



214 nm

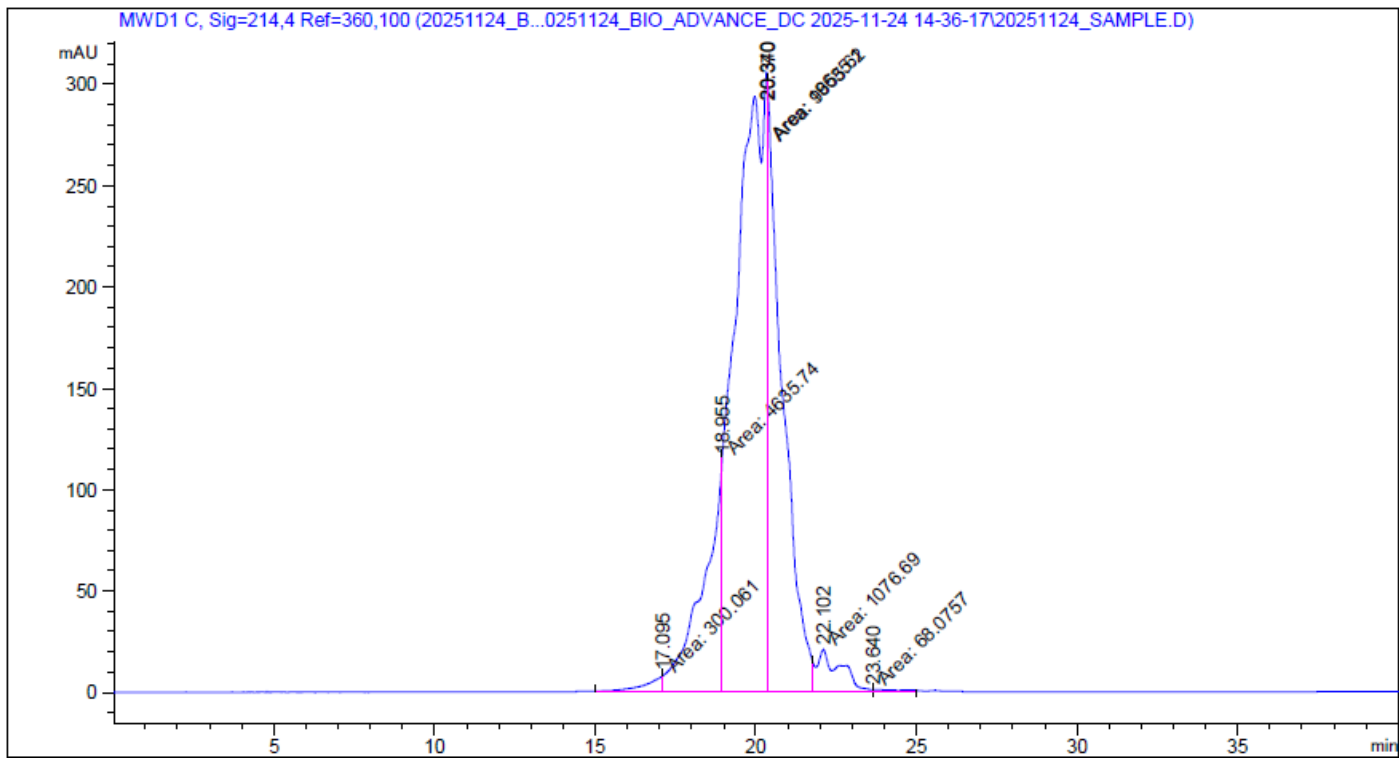
Retention time (min)	Area	% Area
11.078	877.7	2.8
11.500	898.4	2.9
12.220	1994.7	6.4
13.282	5427.6	17.4
15.022	21722.9	69.5
15.657	292.2	0.9
20.532	48.728	0.12

280 nm

Retention time (min)	Area	% Area
11.111	47.7	3.3
11.508	42.1	2.9
12.228	92.8	6.4
13.284	245.6	16.9
15.021	967.5	66.7
15.673	18.8	1.3
20.527	35.030	2.4

Test Sample 2 (APAF Sample ID – n/a)

Retention time-based integration



Peak	MW	Retention time (min)	Area	% Area
1	> 25 kDa	17.095	300.1	0.8
2	25 – 10 kDa	18.955	4635.7	13.1
3	10 – 5 kDa	20.340	19635.1	55.5
4	5 - 2.5 kDa	20.370	9655.6	27.3
5	2.5-1 kDa	22.102	1076.7	3.0
6	< 1 kDa + retained peptides	23.640	68.1	0.2

OPINIONS AND INTERPRETATIONS

Interpretation and/or detailed discussions may be required to fully understand the results presented to you. APAF is committed to assist our clients/collaborators to maximise the value from their results through these consultations. It should be noted that if these results are to be incorporated into a publication, then APAF will be pleased to supply further details/methodology as required by the publishing journal.