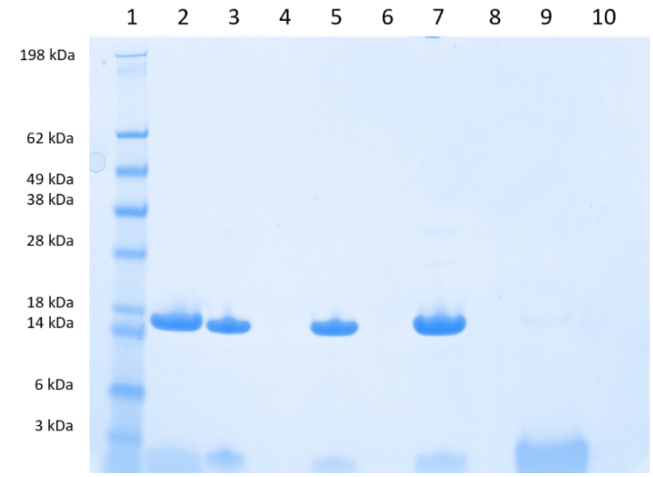
Supporting data	Ab Biotechnology Limited

Figure 1. SDS-PAGE analysis of purified IFNγ



Lane	Sample
1	Marker
2	IFNγ post-DEAE column (~3µg)
3	Concentrated IFNγ, 2.1mg/mL (~3µg)
4	Blank
5	Filtered IFNγ, 1mg/mL (~3µg)
6	Blank
7	Filtered IFNγ, 1mg/mL (~6µg)
8	Blank
9	DEAE column NaCl wash
10	Blank

N-terminal sequencing

The purified, lyophilised IFNγ was analysed by N-terminal sequencing. The first 20 amino acids of the expected sequence were detected (MQDPYVKEAE NLKKYFNAGH) confirming ID.

Mycoplasma testing

Mycoplasma was not detected

Endotoxin testing

A gel clot endotoxin assay indicated an endotoxin level of < 0.5 EU/mL. Endotoxin testing was also carried out using an Endosafe PTS cartridge. The endotoxin concentration was 0.098 EU/mg (0.000098 EU/µg).

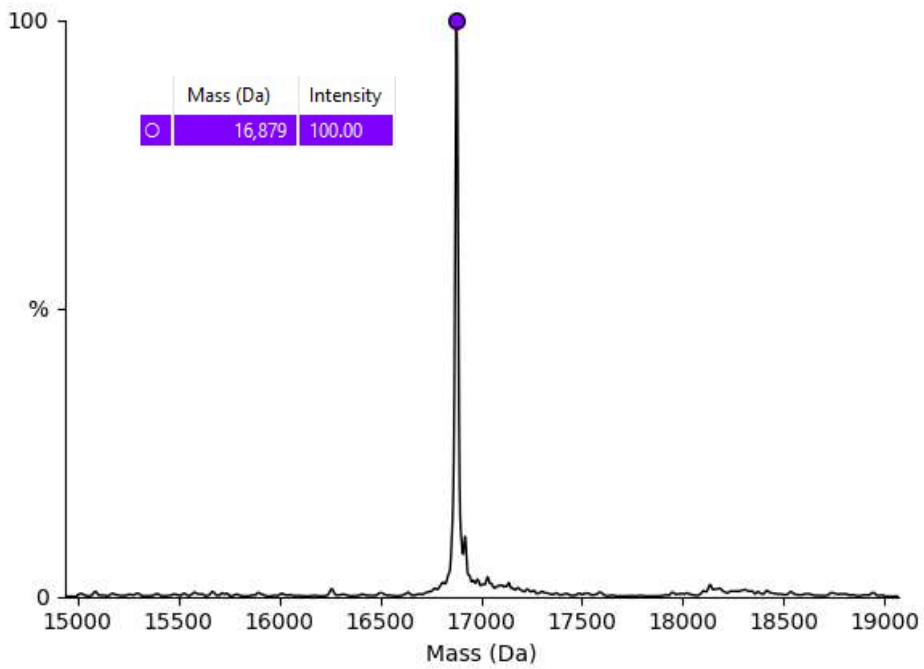
Host cell protein testing

Host cell protein analysis was carried out using an Abcam ELISA kit which gave a value of 0.47 ng per µg of protein.

Mass spectrometry analysis

The intact mass of the IFNγ was determined by LC-Q-ToF mass spectrometry and the result is shown in Figure 2 below. The mass was 16879 Da.

Figure 2. Mass spectrometry analysis of purified IFNγ

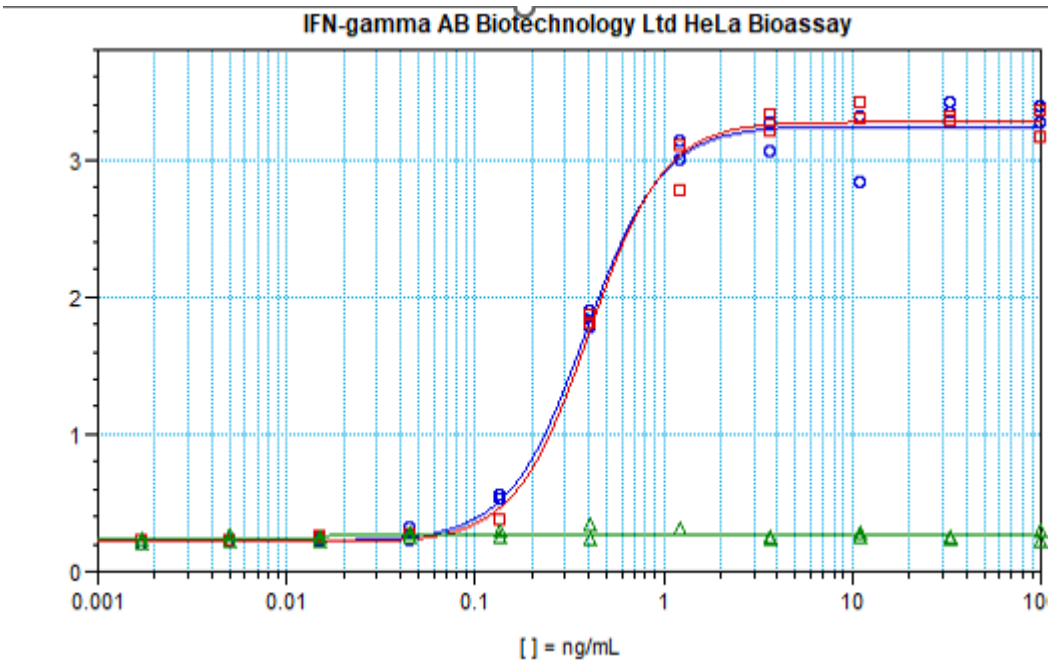


Activity assay

The purified, lyophilised IFNγ was analysed in a bioassay by measuring its ability to protect HeLa human cervical epithelial carcinoma cells from encephalomyocarditis (EMC) viral lysis. TThe ED_{50} specification for the assay is 0.15 – 0.75 ng/mL. Figure 3 below shows that the met the specification

Figure . Bioassay results

Sample	ED_{50} (ng/mL)
E. coli Derived Interferon Gamma Protein	0.726
Buffer/Blank	N/A


IFN-gamma AB Biotechnology Ltd HeLa Bioassay
3
2
1
0
0.001
0.01
0.1
1
10
[ ] = ng/mL