UV Assay of Uricolytic Activity In Fractions from Mono Q Chromatography of PKS Uricase

(Protein Based on Size-Exclusion HPLC)

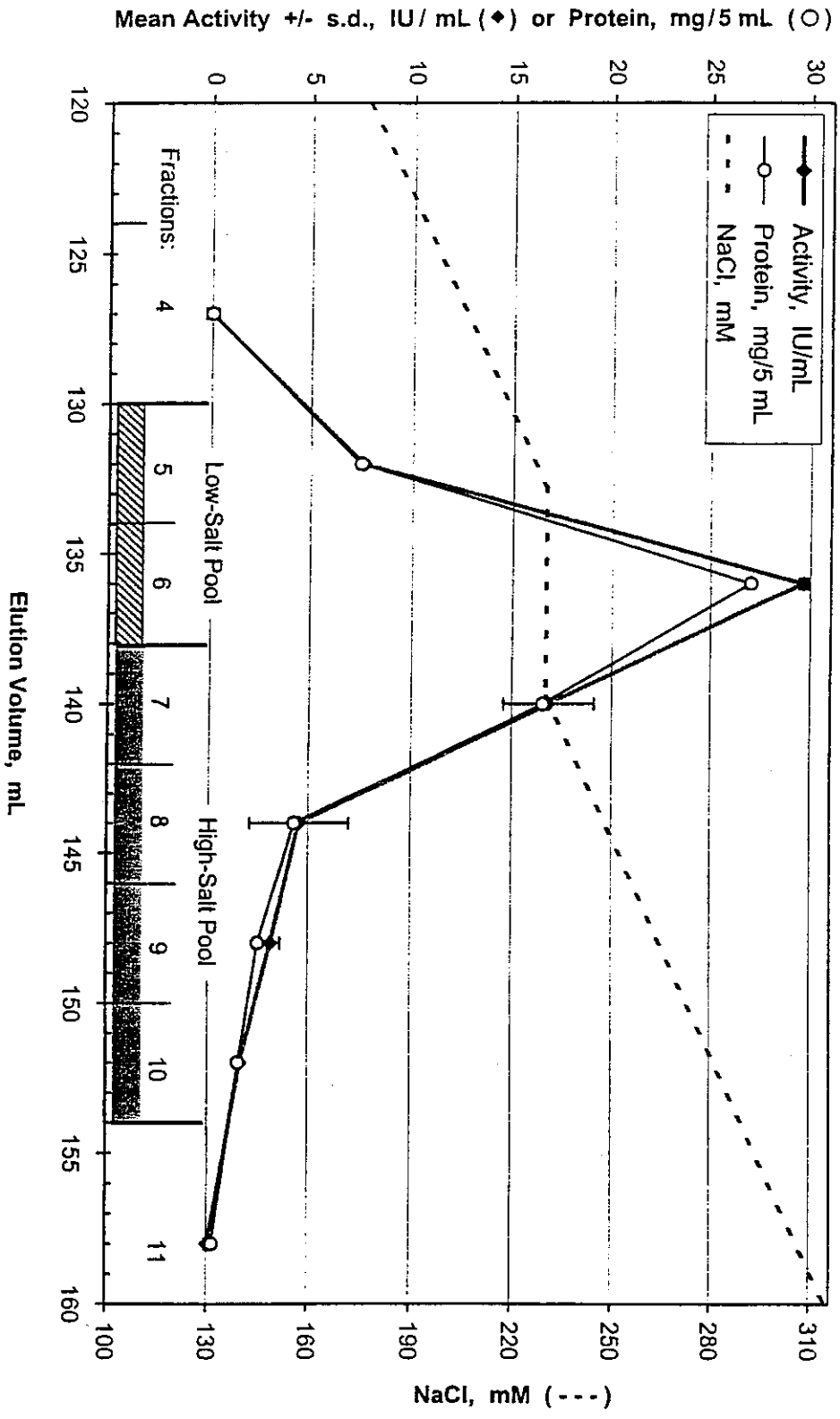


Figure 1

Figure 2

Size-Exclusion HPLC on Superdex 200 of Unfractionated PKS Uricase (Load) and Mono Q Column Fractions in the Low-Salt Pool

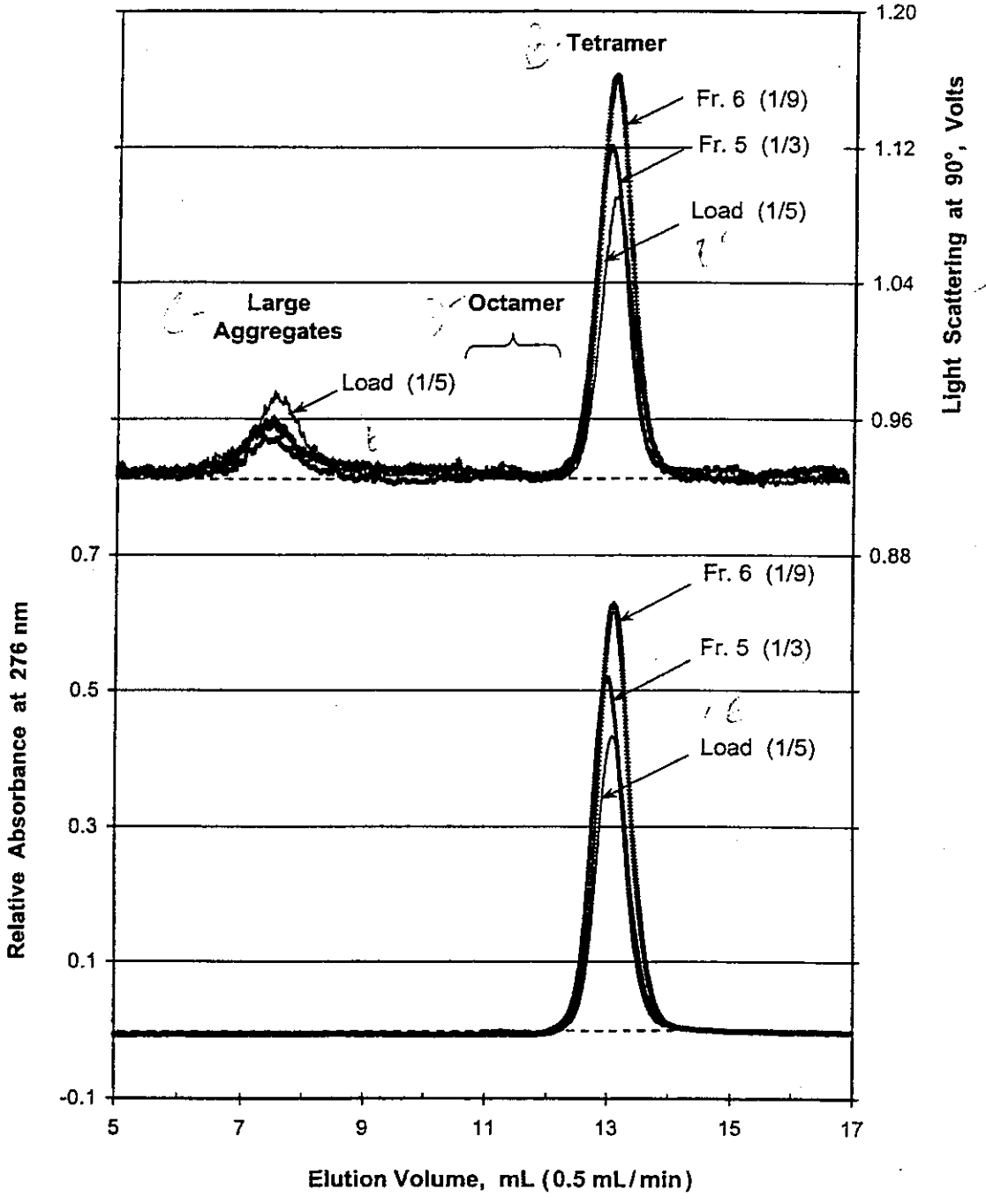
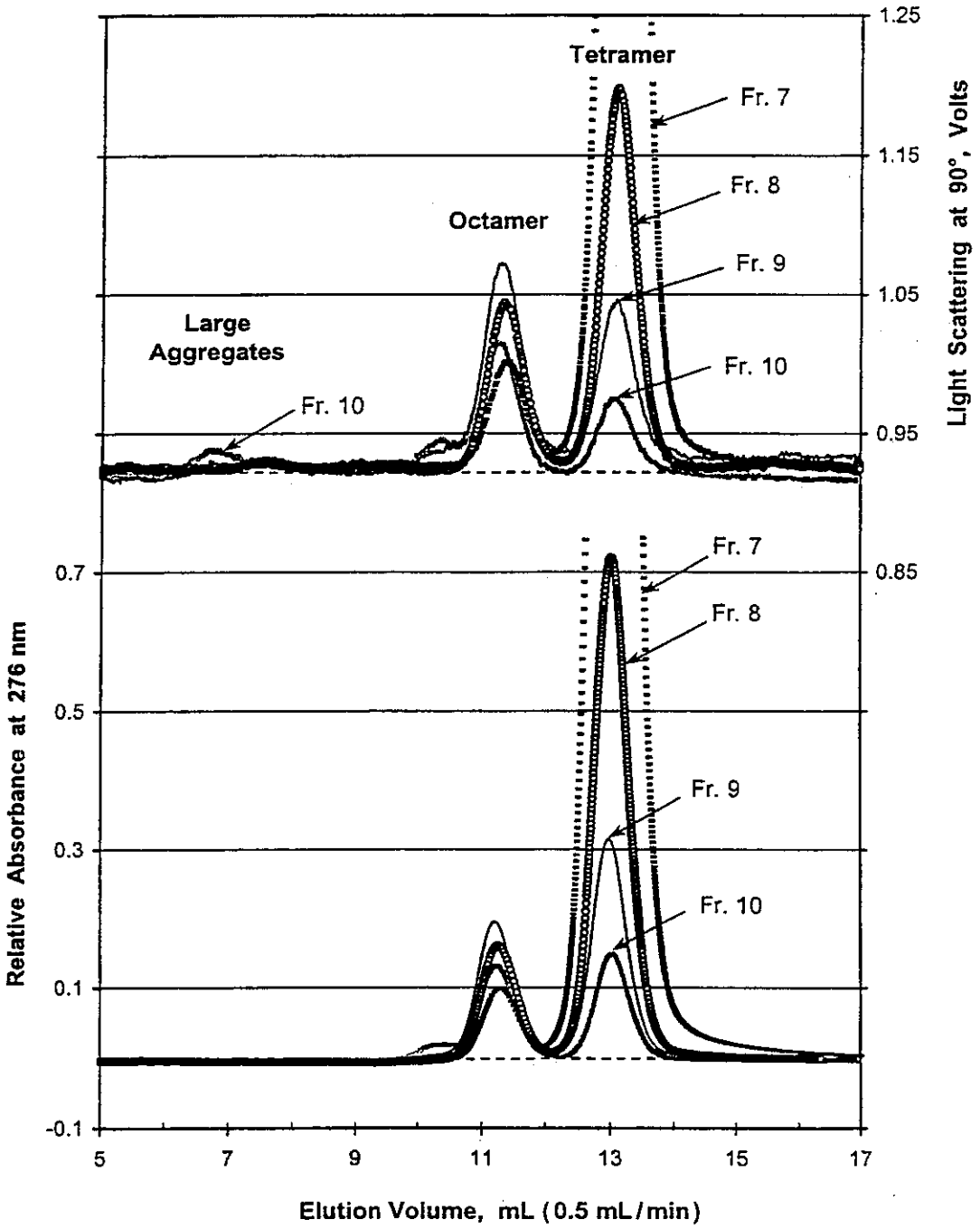
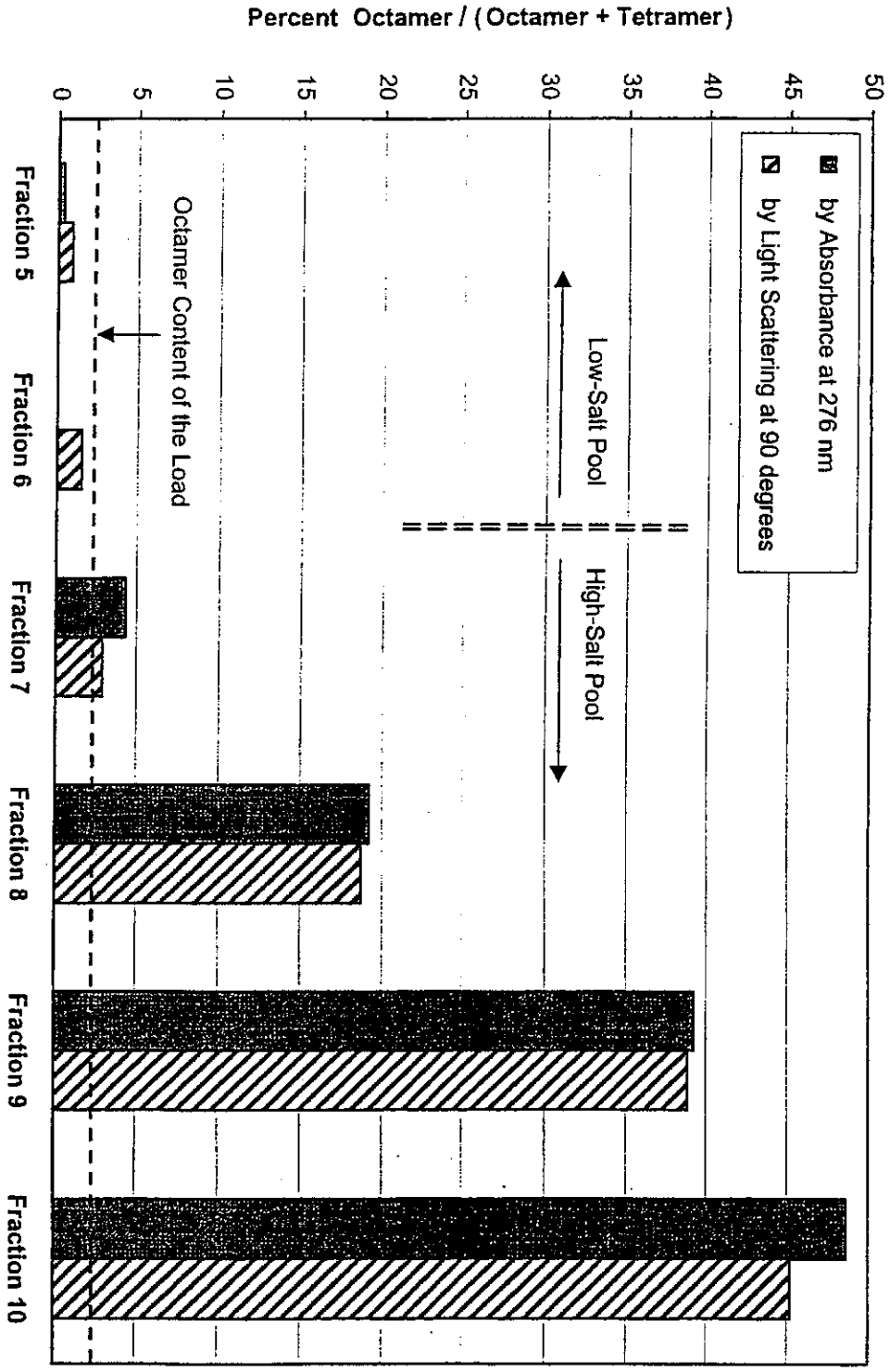


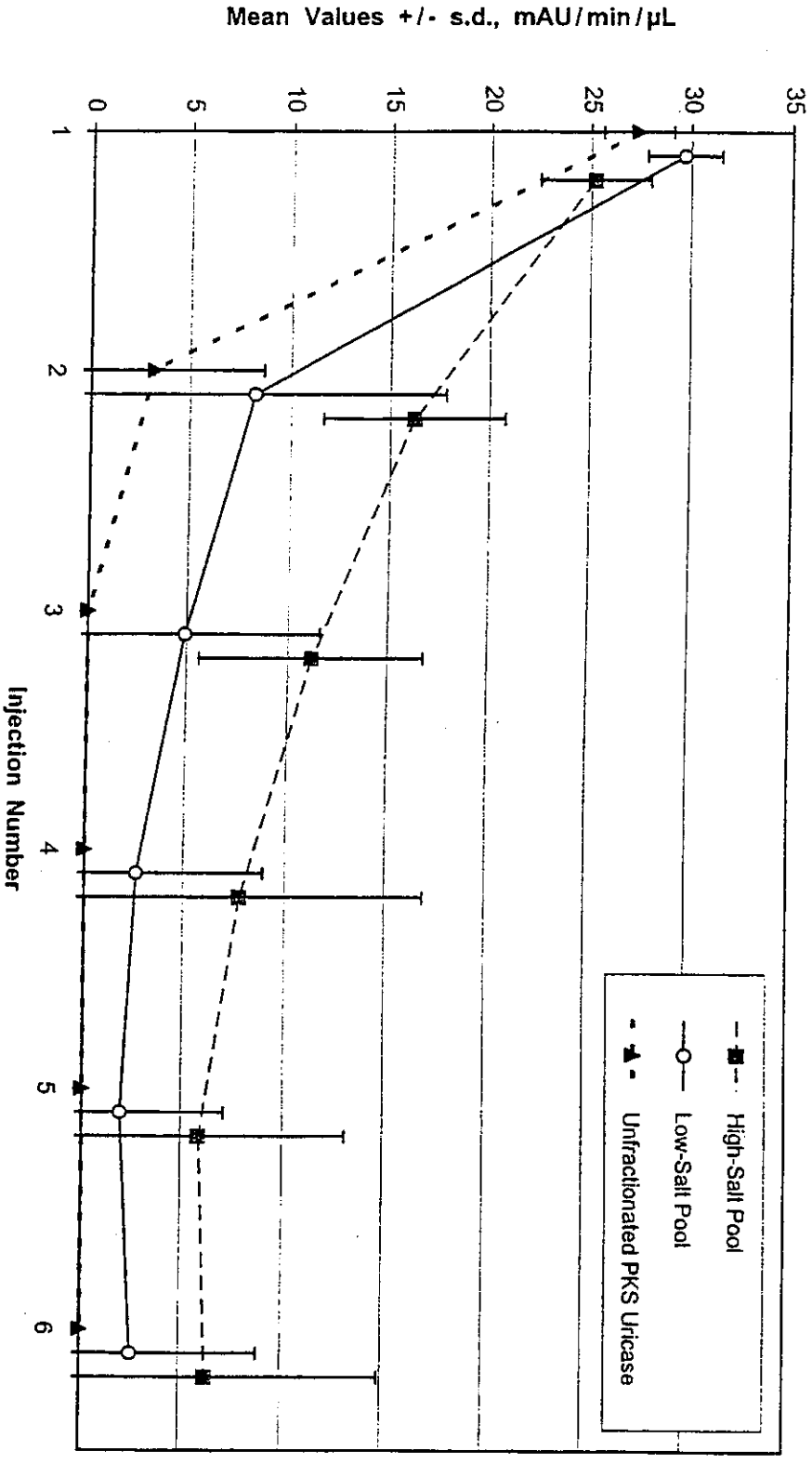
Figure 3


Size-Exclusion HPLC on Superdex 200 of Mono Q
Column Fractions of PKS Uricase in the High-Salt Pool
Tetramer
Octamer
Large
Aggregates
Fr. 7
Fr. 8
Fr. 9
Fr. 10
Fr. 10
Fr. 7
Fr. 8
Fr. 9
Fr. 10
1.25
1.15
1.05
0.95
0.85
Light Scattering at 90°, Volts
0.7
0.5
0.3
0.1
-0.1
Relative Absorbance at 276 nm
5
7
9
11
13
15
17
Elution Volume, mL (0.5 mL/min)



Octamer Content of Mono Q Column Fractions of PKS Uricase

Figure 4



UV Uricase Assays of Sera from Mice Injected with 6 x 10-kDa PEG Conjugates of PKS Uricase or of Pools from Mono Q Column Fractions

Mice Were Bled 24 Hours after Each Weekly Injection.

Data for the Low-Salt and High-Salt Pools were shifted on the x-axis by 0.1 and 0.2 units, respectively.

Figure 5

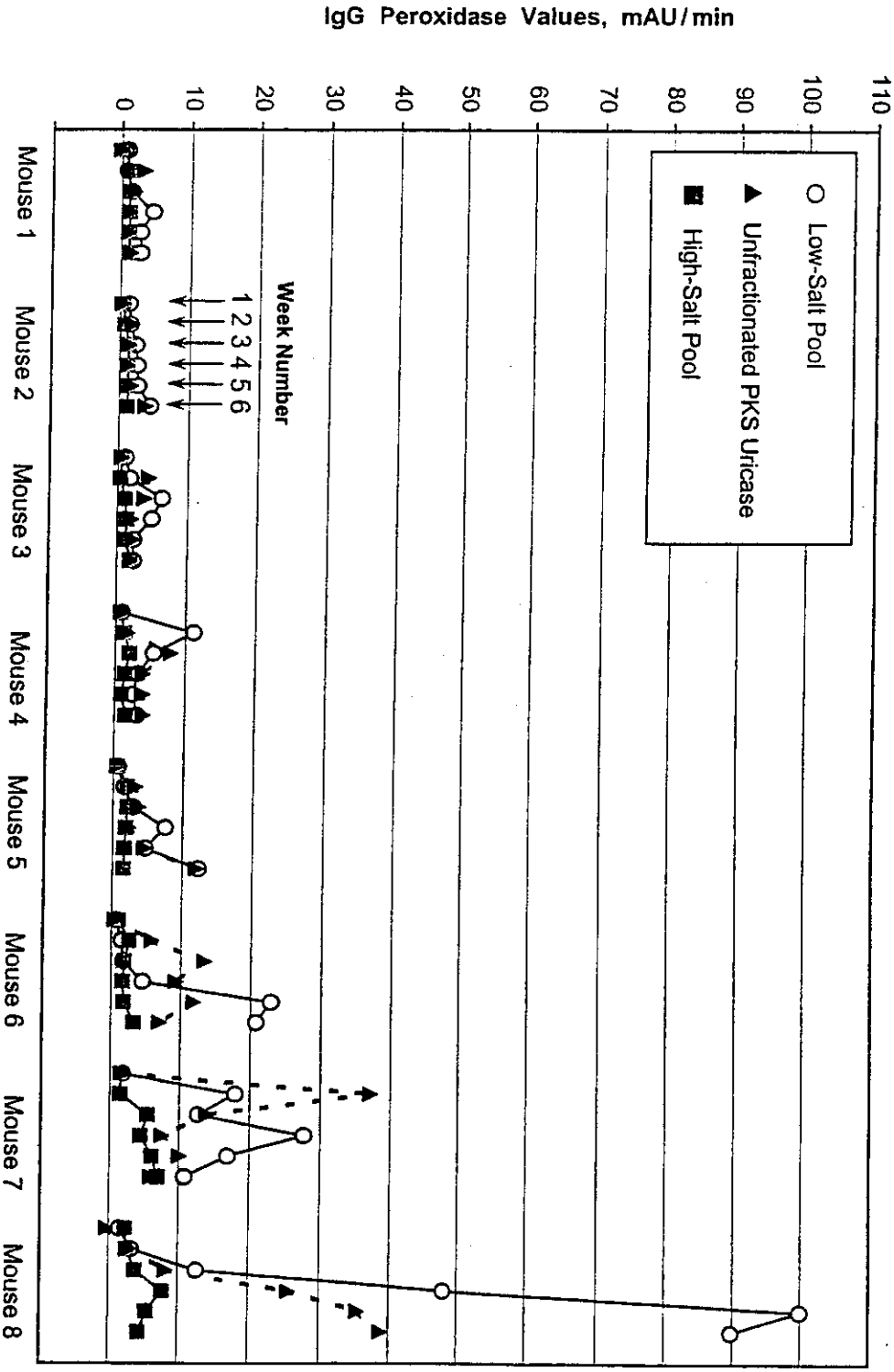

ELISA Analyses with Pig Uricase on the Plate and 1:30 Sera from Mice Injected with
6 x 10-kDa PEG Conjugates of Unfractionated PKS Uricase or Low-Salt or High-Salt Pools
IgG Peroxidase Values, mAU/min
Low-Salt Pool
Unfractionated PKS Uricase
High-Salt Pool
Week Number
1 2 3 4 5 6
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8

Figure 6