Comparison of MW determined by "two detector" approximation and ASTRA analysis

Molecular Weight Determined from "two detector" analysis

Protein	Oligomeric state	predicted MW (kDa)	number of runs	Average Experimental MW (kDa)	SD[a)] (kDa)	Average difference[b)] (%)	comments
Alcohol Dehydrogenase	tetramer	147.5	4	145.8	1.5	1.2	
BSA (monomer)	monomer	66.4	5	67.1	0.9	1.2	
BSA (dimer)	dimer	132.9	5	140.6	2.8	5.8	
Carbonic Anhydrase	monomer	29.0	4	29.8	1.0	3.8	
Cytochrome C	monomer	12.3	5	11.9	0.2	2.9*	*colored**
Apo-ferritin	24*monomer	475.9	2	469.8	0.3	1.3	
Alfa-Lactalbumin	monomer	14.2	2	15.1	0.03	6.8	
Aldolase (rabbit)	tetramer	156.8	1	150.6		2.2	
Beta-lactglobulin	monomer +dimer	18.3	2	21.5	0.5	17.5	*known to dimerize at low pH*
Enolase (rabbit)	dimer +monomer	93.7	4	86.9	1.5	7.2**	*needs Mg2+ for dimer stability*
Enolase (yeast)	dimer +monomer	93.3	1	80.1		14.2**	*needs Mg2+ for dimer stability*
Glutamate Dehydrogenase	hexamer	333.4	6	461.6	44.2	38.5	*"tailing" polydisperse peak*
Myoglobin	monomer	17.0	3	14.2	1.6	16.2*	*colored**
Transferrin	monomer	75.2	2	78.0	0.3	3.8	
Trypsin Inhibitor	monomer	20.0	1	21.0		5.0	
Ovalbumin	monomer	42.8	10	43.6	0.5	1.9	

Buffer used: 20 mM HEPES, 100 mM KCl, 1 mM EDTA pH=8.0 @ RT

a) SD represents one standard deviation calculated as S.D. = SQRT{ [sum(Yi-M)^2]/(n-1)}, where
M is arithmetic mean caluclated as M = sum(Yi)/n (given in column "Average Experimental MW")
Yi is a result of the "ith" measurement , *i.e.* MW determined in the "ith" run
n is a number of runs

b) Average difference (%) is calculated as absolute value of {100*[(Average Experimental MW-predicted MW)/predicted MW]}

* *colored proteins absorb at the wavelength of the laser beam (633 nm). Thus the amount of scattered light is smaller and leads to underestimated Mw as the instrument is not capable of correcting for the absorbed light.*

** *these dimers are unstable under chromatographic condition used, i.e. buffer with 1 mM EDTA.*

Molecular Weight Determined from ASTRA analysis

Protein	Oligomeric state	Predicted MW (kDa)	Number of runs	Average Experimental MW (kDa)	SD (kDa)	Average difference (%)	Comments
Alcohol Dehydrogenase	tetramer	147.5	4	144.0	0.9	2.4	
BSA (monomer)	monomer	66.4	5	67.1	1.0	1.2	
BSA (dimer)	dimer	132.9	5	137.1	3.9	3.2	
Carbonic Anhydrase	monomer	29.0	4	29.2	0.2	0.8	
Cytochrome C	monomer	12.3	5	12.0	0.6	2.4*	*colored**
Apo-ferritin	24*monomer	475.9	2	470.4	2.6	1.2	
Alfa-Lactalbumin	monomer	14.2	2	14.3	0.01	0.9	
Aldolase (rabbit)	tetramer	156.8	1	155.1		1.1	
Beta-lactglobulin	monomer +dimer	18.3	2	20.1	0.3	9.7	*known to dimerize at low pH*
Enolase (rabbit)	dimer +monomer	93.7	4	86.4	1.9	7.8**	*needs Mg2+ for dimer stability*
Enolase (yeast)	dimer +monomer	93.3	1	79.5		14.9**	*needs Mg2+ for dimer stability*
Glutamate Dehydrogenase	hexamer	333.4	6	486.5	48.7	45.9	*"tailing" polydisperse peak*
Myoglobin	monomer	17.0	3	14.2	0.9	16.3*	*colored**
Transferrin	monomer	75.2	2	76.9	1.0	2.3	
Trypsin Inhibitor	monomer	20.0	1	20.5		2.3	
Ovalbumin	monomer	42.8	10	42.5	0.7	1.4	

Buffer used: 20 mM HEPES, 100 mM KCl, 1 mM EDTA pH=8.0 @ RT

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