

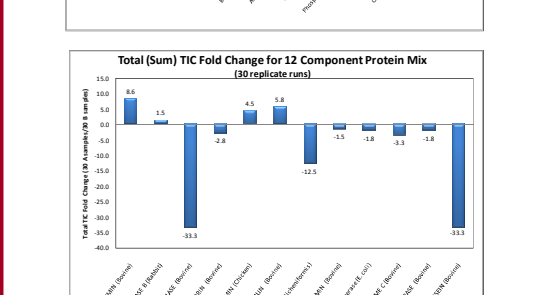
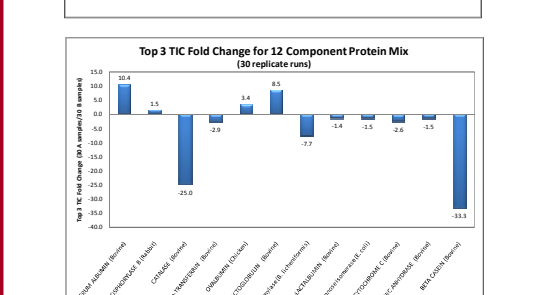
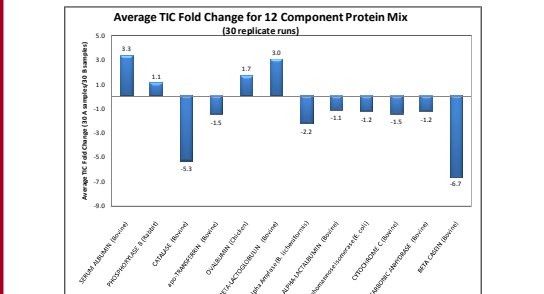
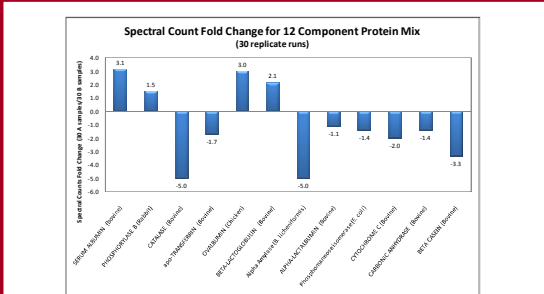
# Using MS/MS Total Ion Current (TIC) Quantification in Scaffold 3 Software - Comparison to Spectral Counting

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While spectral counting is an easy and straightforward method to semi-quantify proteins based on shotgun LC/MS/MS spectra, use of the total ion current (TIC) from each identified MS/MS spectra has advantages such as increased dynamic range (1) and quantification for low spectral counts. Here, we evaluate different forms of TIC: Average, Total (Sum) and Top3 for shotgun proteomics data analysis using a 12-component protein mixture of varying concentrations (2) and applications to protein-protein interaction (PPI) studies in human cancers. We also show that Average TIC may be effective for determining the relative *stoichiometry of a protein complex* from a single sample since ionization differences are averaged across multiple peptides.

Screenshot from a portion of Scaffold 3.1 software showing the TIC options under the Quantitative Analysis Tab from the Experiment drop-down menu. Scaffold now allows for the use of Average TIC, Top3 TIC and Total (Sum) TIC in addition to spectral counting.



Fold change (mix A/mix B) based on Spectral counting, Average TIC, Top3 TIC and Total (Sum) TIC from all identified MS/MS spectra per protein in mixture for 30 replicate analyses per mixture. Notice that the quantitative trend for all methods is similar to the fold change based on true concentrations (left), however, the fold change values vary between analyses. A baseline TIC value of 1 x10E4 was used if no data was acquired for some samples.

Protein	Fold Change (A sample / B sample), CV % A and B											
	Spectral Counts	CV	CV	Average TIC	Avg TIC	Top 3 TIC	Top3 TIC	Sum TIC	Sum TIC	CV (A)	CV (B)	
SERUM ALBUMIN (Bovine)	12.0	3.1	37	15	3.3	46	51	10.4	21	7	8.6	2
PHOSPHORYLASE B (Rabbit)	2.0	1.5	18	23	1.1	39	71	1.5	32	57	1.5	23
CATALASE (Bovine)	-12.0	-5.0	22	12	-3.3	37	58	-25.0	53	23	-33.3	29
apo-TRANSFERRIN (Bovine)	-2.0	-1.7	9	1	-1.5	24	44	-2.9	27	30	-2.8	25
OVAALBUMIN (Chicken)	6.0	3.0	14	22	1.7	63	93	3.4	55	64	4.5	31
BETA-LACTOGLOBULIN (Bovine)	9.0	2.1	11	17	3.0	44	58	8.5	35	40	5.8	27
Alpha Amylase B (Iicheniformis)	-5.0	-5.0	18	28	-2.2	26	35	-7.7	33	61	-12.5	37
ALPHA-LACTALBUMIN (Bovine)	1.5	-1.1	25	27	-1.1	58	96	-1.4	42	112	-1.5	29
Phosphomannose Isomerase (E. coli)	1.0	-1.4	26	26	-1.2	23	61	-1.5	35	40	-1.8	39
CYTOCHROME C (Bovine)	3.0	-2.0	44	35	-1.5	43	53	-2.6	54	43	-3.3	53
CARBONIC ANHYDRASE (Bovine)	1.0	-1.4	29	37	-1.2	67	94	-1.5	367	375	-1.8	356
BETA-CASEIN (Bovine)	-9.0	-3.3	18	17	-6.7	24	137	-33.3	36	143	-33.3	38

The fold change accuracy results show that each TIC quantification method and spectral counting correlate well with known concentrations for some proteins in mix but fail for other proteins. According to these data, both Spectral Counting and Average TIC show the most accurate results. (Green-close to actual fold change; Orange-near actual fold change; Magenta-far from actual fold change). Average coefficient of variation (CV,%) across different MS/MS based label-free quantitative methods for mixtures A and B. Mixture B showed more variation across 30 runs than mixture A. Spectral counting shows lower CV values than the TIC methods, however, this could in part be due to the fact the spectral counting shows lower dynamic range.

### Stoichiometry Evaluation of The 12-Component Protein Mix by Average TIC

Protein	A Conc. (pmol)	B Conc. (pmol)
SERUM ALBUMIN (Bovine)	10	2
PHOSPHORYLASE B (Rabbit)	2	1
CATALASE (Bovine)	1	12
apo-TRANSFERRIN (Bovine)	1	2
OVALBUMIN (Chicken)	6	1
BETA-LACTOGLOBULIN (Bovine)	9	1
Beta-Casein (Bovine)	1	6
ALPHA-LACTALBUMIN (Bovine)	9	6
Phosphomannose Isomerase (E. coli)	1	1
CYTOCHROME C (Bovine)	1	1
CARBONIC ANHYDRASE (Bovine)	3	1
BETA-CASEIN (Bovine)	1	9

Average TIC may be used to quantify the stoichiometry of protein complexes within a sample without isotope labels. Analysis of the 12-protein mixture indicates correlation with known concentrations.

### Evaluation of a 12 Protein Mixture of Known Concentrations

Protein	Mixture A		Mixture B			
	Ratio	pmol	Ratio	pmol		
Serum Albumin (Bovine)	12	831.69	41.81	1	52.63	2.48
Carbonic Anhydrase (Bovine)	3	157.69	4.88	1	52.63	1.63
Catalase (Bovine)	1	52.03	3.10	12	631.08	37.89
Cytocytome C (Bovine)	1	52.03	2.22	1	52.63	2.22
Egg White Albumin (Chicken)	6	315.79	13.89	1	52.63	2.33
Phosphorylase B (Rabbit)	2	105.26	10.23	1	52.63	5.12
apo-Transferrin (Bovine)	1	52.63	4.11	2	105.26	8.21
alpha-Amylase B (Iicheniformis)	1	52.63	2.89	6	315.79	17.37
beta-Casein (Bovine)	1	52.63	1.32	9	473.68	11.84
beta-Lactoglobulin (Bovine)	9	473.68	8.72	1	52.63	0.97
alpha-Lactalbumin (Bovine)	9	473.68	6.72	6	315.79	4.48
Phosphomannose Isomerase (E. coli)	1	52.63	2.25	1	52.63	2.25
		102.31				97.80

The protein components of the 12 component mixture showing the actual concentrations used for 30 replicate shotgun LC/MS/MS analyses for both mixtures A and B using an LTQ linear ion trap.

The mix A / mix B fold change based on known concentrations in each mixture. This is the true reference for MS comparison.

### Stoichiometry of PI3K Subunits in Prostate Cancer Cell Lines by Average TIC

•MW of each component is not important (not true for spectral counting)  
 •High spectral count value is less important  
 •Peptide ionization differences are less important since the signal averages across many peptides  
 •No isotope labels necessary

Western blot validation

### Quantification of Activating PI3K Adaptors in NSCL Cancer by Average TIC

Both Spectral Counting and Average TIC show a decrease in p85-ERBB3 binding upon the EGFR inhibitor (gefitinib) treatment, which results in decreased pAKT activity and tumor shrinkage in EGFR mutated non-small cell lung cancers (NSCL). HGF + gefitinib eliminates p85-ERBB3 association but activates PI3K/AKT through other adaptors such as IRS1/2 and GAB1/2. p85(P13K) was immunoprecipitated (IP) and run by shotgun LC/MS/MS using a LTQ Orbitrap XL mass spectrometer.

1. Asara JM, Christofk HR, Freemark LM, Cantley LC. A label-free quantification method by MS/MS TIC compared to SILAC and spectral counting in a proteomics screen. *Proteomics*. 2008; 8:994-9.  
 2. Data from label-free shotgun runs courtesy of John Klimek and Larry David, Oregon Health & Science University (2008, ASMS, poster)