April 7, 2017

Client Company 0000 Main St. Madison, WI 53716

Dear Client:

Results of the electrophoretic analysis of your two samples received 4/4/17 are presented on the following pages. Figures 1 and 2 depict protein band location, percent of total protein for each of the major bands in the samples relative to total stain density per lane, and molecular weights for the non-reduced 10% acrylamide slab gel MH p.426 #2. Figure 3 shows an image of the gel; Table 1 describes the gel loading scheme and lane identifications. Materials and Methods are described below. Also included is the original dried gel.

## Methods

The protein concentrations of the samples were determined by the BCA assay (Smith et. al. *Anal. Biochem. 150:* 76-85, 1985, and Pierce Chemical Co., Rockford, IL).

The samples were diluted with sample buffer containing 5.0% sodium dodecyl sulfate (SDS), 10% glycerol, 50 mM dithiothreitol, and 63 mM tris, pH 6.8. Following buffer addition, the samples were heated in a digital dry bath at 96°C for 10 minutes.

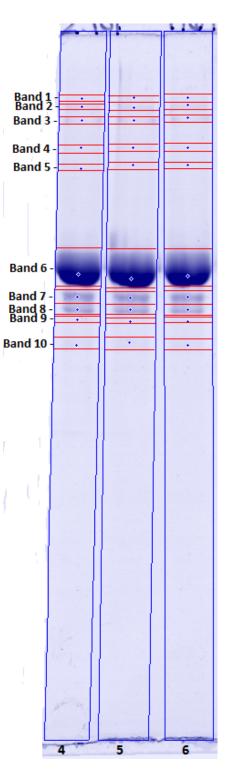
SDS slab gel electrophoresis was carried out according to the method of Laemmli (Laemmli, U.K. *Nature* 227: 680-685, 1970) as described by Burgess-Cassler et. al. (*Clin. Chem.* 35:2297-2304, 1989; second dimension) using 10% acrylamide slab gels (125mm length X 150mm width X 0.75mm thickness) overlaid with a 25 mm stacking gel. Electrophoresis was performed using 15 mAmp/gel for about 3.5 hours at which time the bromophenol blue front had migrated to the end of the slab gels. The gels were stained with Coomassie blue, destained in 10% acetic acid until a clear background was obtained, then dried between cellophane sheets.

The following proteins (Sigma Chemical Co., St. Louis, MO, and EMD Millipore, Billerica, MA) were added as molecular weight standards to a well: myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000).

Sincerely,

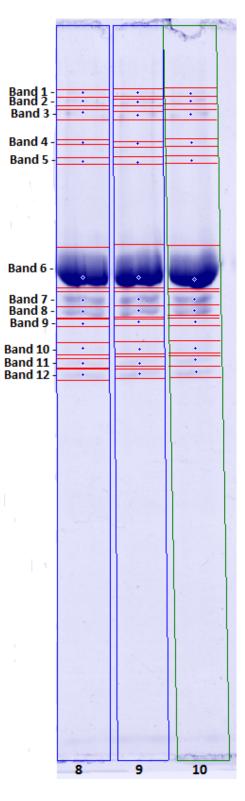
Keith P. Oberle Senior Biochemist

Band	Ave. Band %	<u>+</u> SD	Mol. Wt. (Da)	
1	0.07	0.05	225,155	
2	0.52	0.13	207,412	
3	0.64	0.12	182,782	
4	0.14	0.08	126,071	
5	0.07	0.08	93,192	
6	87.22	0.50	55,129	
7	6.91	0.01	47,971	
8	4.26	0.06	43,596	
9	0.02	0.03	41,952	
10	0.15	0.07	39,145	



**Figure 1**. **Sample: Product A.** Stain density in individual protein bands as a percentage of total stain per lane. The image above shows lanes 4, 5, and 6 from gel MH p.426 #2 with the proteins labeled.

	<b>A</b> = + a		$M_{c1}$ W/	
Band	Ave.	<u>+</u> SD	Mol. Wt.	
	Band %		(Da)	
1	0.08	0.02	225,108	
2	0.42	0.07	209,216	
3	0.50	0.14	185,378	
4	0.02	0.02	130,324	
5	0.02	0.02	96,270	
6	86.12	0.59	55,851	
7	6.45	0.12	48,667	
8	4.56	0.18	44,720	
9	0.05	0.08	42,319	
10	0.27	0.18	39,287	
11	0.49	0.09	37,822	
12	1.03	0.12	36,494	



**Figure 2**. **Sample: Product B.** Stain density in individual protein bands as a percentage of total stain per lane. The image above shows lanes 8, 9, and 10 from gel MH p.426 #2 with the proteins labeled.

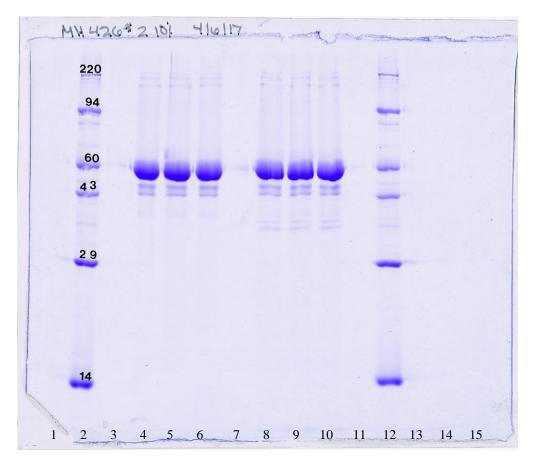
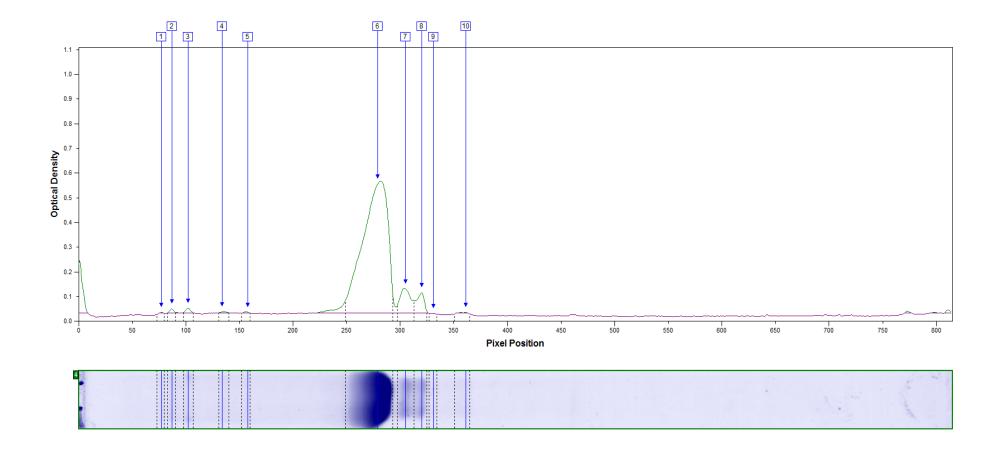


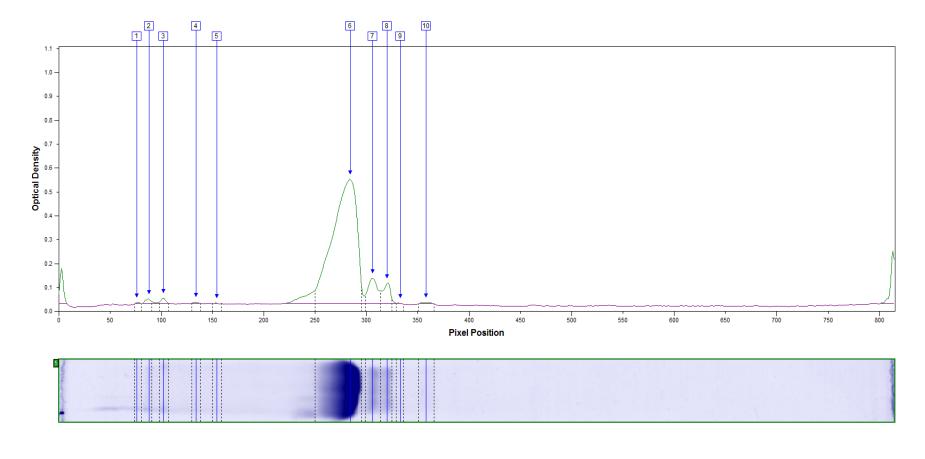
Figure 3. Image of gel MH p.426 #2.

Lane	Sample	u a Dratain	ul loodod
Lalle	Sample	μg Protein	µl loaded
1	-	-	-
2	Molecular Weight Standards	-	5
3	SDS Buffer without BME	-	10
4	Product A	5	10
5	Product A	5	10
6	Product A	5	10
7	-	-	-
8	Product B	5 + 0.1	10 +
9	Product B	5 + 0.1	10 +
10	Product B	5 + 0.1	10 +
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-

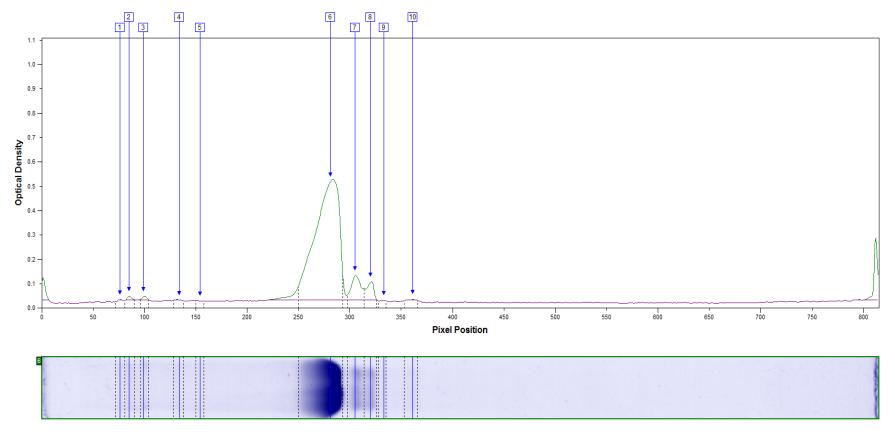
**Table 1**. Key to Loading gel MH p.426 #2.



Gel MH p.426 #2 Lane 4 Sample: Product A

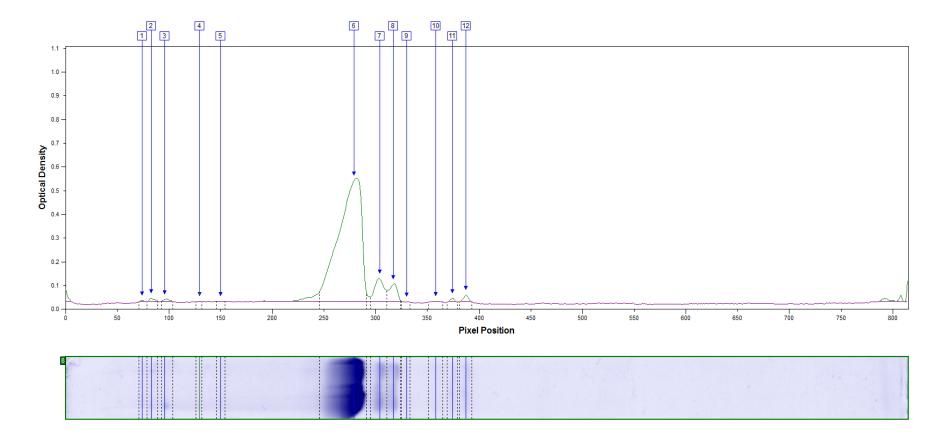


Gel MH p.426 #2 Lane 5 Sample: Product A

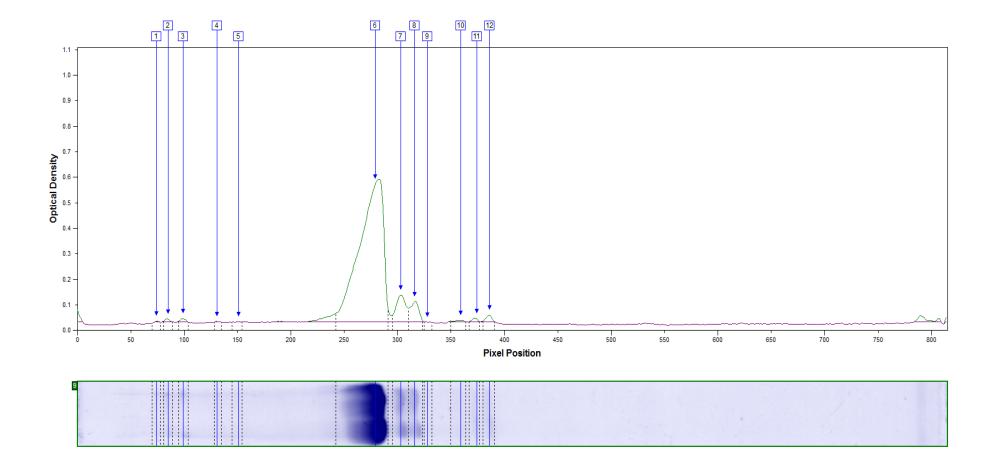


Gel MH p.426 #2

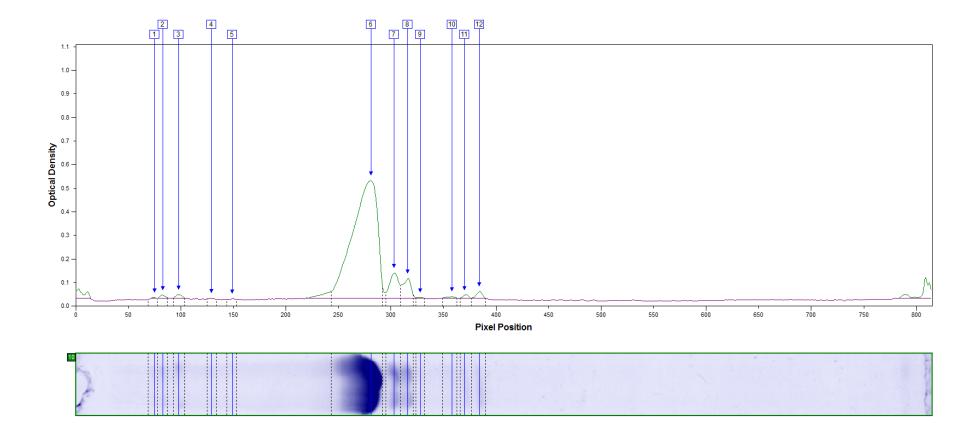
Lane 6 Sample: Product A



Gel MH p.426 #2 Lane 8 Sample: Product B



Gel MH p.426 #2 Lane 9 Sample: Product B



Gel MH p.426 #2 Lane 10 Sample: Product B