January 3, 2016

Ms. Client
Biology Company
0000 Main St.
Madison, WI 53715

Dear Client:

Results from the electrophoretic analysis of your two samples received 1/1/16 are enclosed.  Figure 1 shows protein band numbering for each of the major bands in the samples. Table 1 lists the percent of total protein relative to total stain density per lane for each of the marked bands in Figure 1. Figure 2 is a gel image with Table 2 describing the gel loading scheme.  Methods are described below. Also included are peak density profile plots and the dried Coomassie blue-stained gels.

**Methods**

Samples were weighed and dissolved to a 5 mg/ml protein concentration with sample buffer containing 5.0% sodium dodecyl sulfate (SDS), 10% glycerol, with 50 mM Dithiothreitol. Following buffer addition, the samples were heated in a didgital dry bath at 95$^{o}$C for 5 minutes before loading.

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 a 12% acrylamide slab gel (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 hrs. at which time the bromophenol blue front had migrated to the end of the slab gel.  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).

The stained gel was digitized over the appropriate optical density range using a calibrated GE Healthcare Image Scanner III.  Stain density in individual protein bands as a percentage of total stain per lane was quantified using Phoretix 1D software (version 11.2) with a Windows 10 compatible computer.

Please feel free to call with any questions or comments.
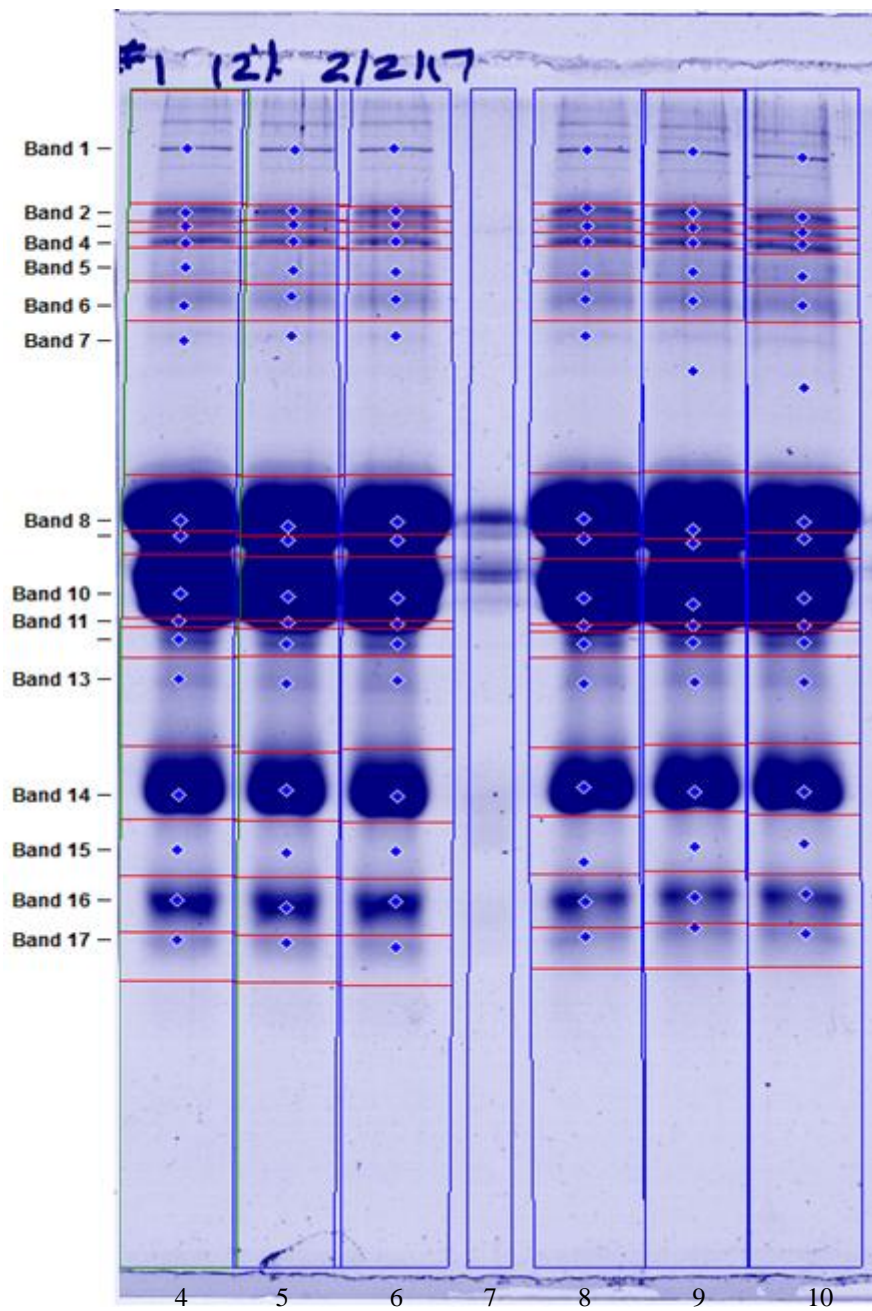
Sincerely,

Lindsey Eierman
Biochemist

Figure 1. Protein band numbering for milk proteins (gel LE p.106#1.) From left to right: lanes 4-6 (NFDM), lanes 8-10 (NFDM + Casein). Each numbered band has a corresponding value in Table 1 representing the percent of total stain per lane within the sample.

| Protein ID | Band | NFDM | | NFDM + Casein | |
|---|---|---|---|---|---|
| | | Ave. | +/- SD | Ave. | +/- SD |
| Unknown | 1 | 1.73 | 0.12 | 2.34 | 0.26 |
| Lactoferrin | 2 | 1.13 | 0.09 | 1.32 | 0.06 |
| Unknown | 3 | 0.65 | 0.06 | 0.87 | 0.05 |
| Bovine Serum Albumin | 4 | 1.07 | 0.02 | 1.19 | 0.04 |
| Unknown | 5 | 0.81 | 0.08 | 1.26 | 0.07 |
| IgG Heavy Chain | 6 | 1.15 | 0.03 | 1.56 | 0.08 |
| Unknown | 7 | 1.09 | 0.15 | 1.28 | 0.08 |
| α s1-casein | 8 | 24.05 | 0.02 | 23.97 | 1.51 |
| α s2-casein | 9 | 10.81 | 0.47 | 11.08 | 1.49 |
| β-casein & IgG Light Chain | 10 | 29.99 | 0.15 | 29.44 | 0.47 |
| γ1-casein | 11 | 2.63 | 0.07 | 2.38 | 0.53 |
| κ-casein | 12 | 1.95 | 0.13 | 1.76 | 0.09 |
| Unknown | 13 | 2.73 | 0.30 | 2.50 | 0.15 |
| β-lactoglobulin | 14 | 14.11 | 0.35 | 13.36 | 0.13 |
| Unknown | 15 | 0.91 | 0.04 | 0.94 | 0.08 |
| α-lactalbumin | 16 | 4.41 | 0.03 | 3.65 | 0.15 |
| Unknown | 17 | 0.70 | 0.05 | 1.08 | 0.08 |

Table 1.  Protein identification and percent of total protein relative to stain density is provided for each of the major bands shown in Figure 1. Values are stain density in individual protein bands as a percentage of total stain per lane +/- standard deviation.  Each value is the average of three lanes.
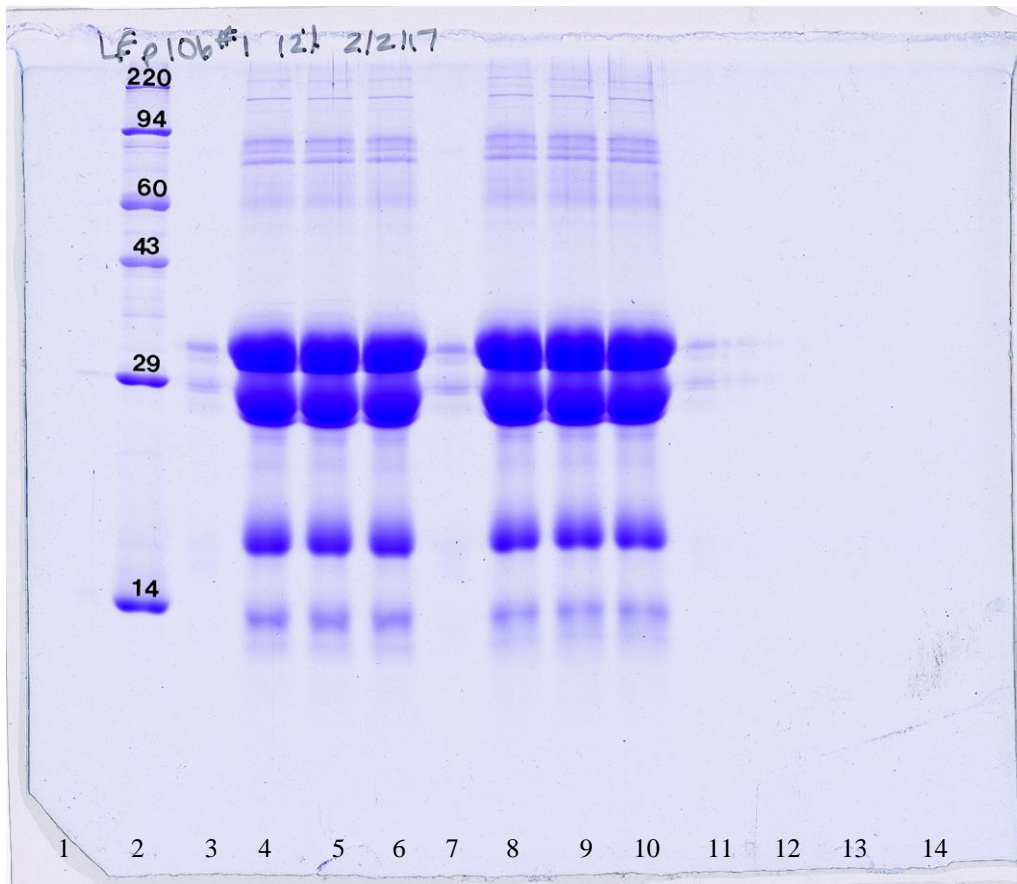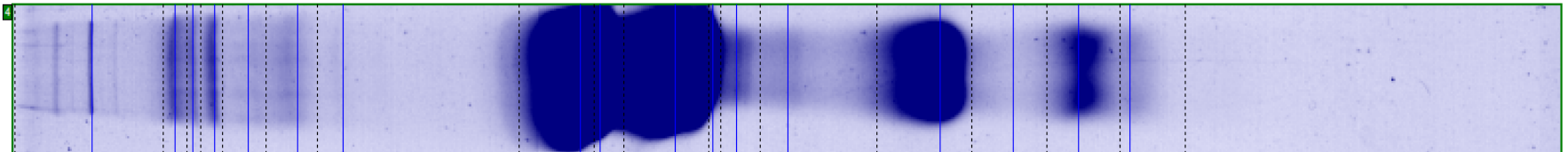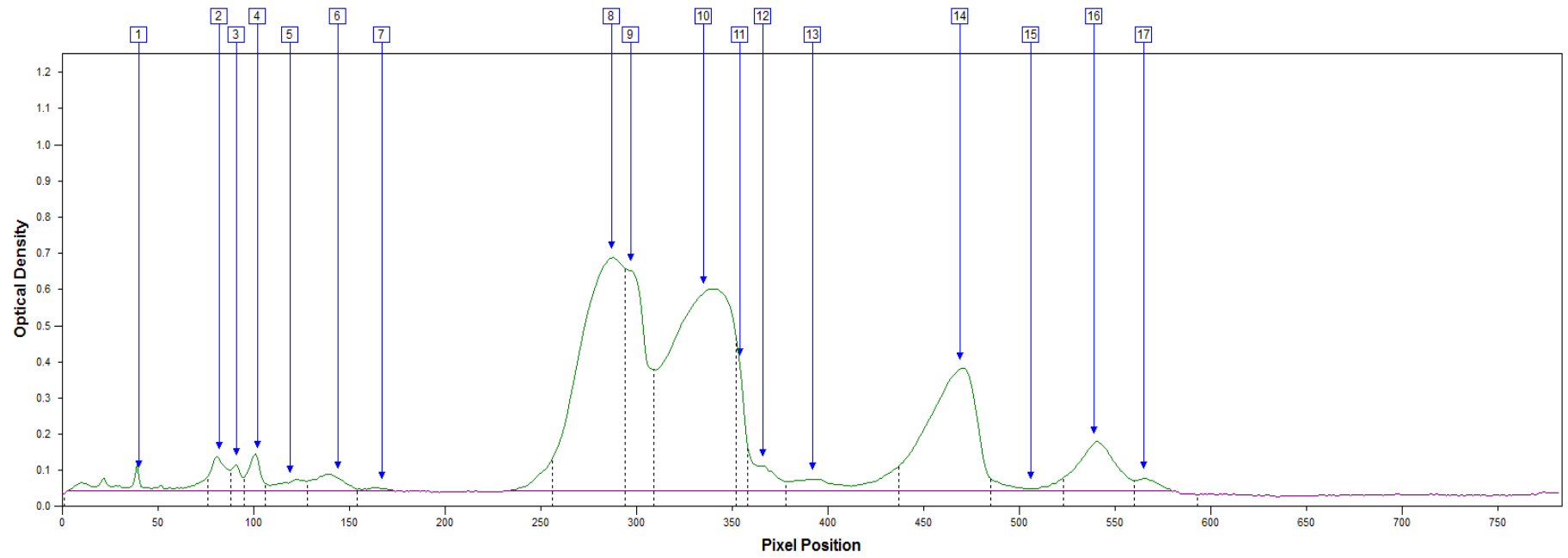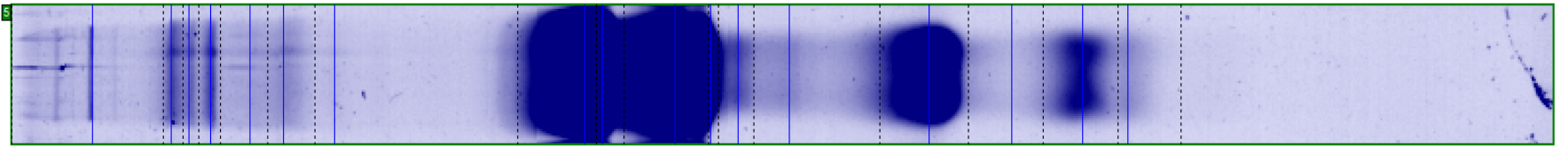
Figure 2. Image of gel LE p.106 #1.

| Lane # | Sample | µg Protein |
|---|---|---|
| 1 | - | |
| 2 | Molecular Weight Standards | |
| 3 | - | |
| 4 | NFDM | 35 |
| 5 | NFDM | 35 |
| 6 | NFDM | 35 |
| 7 | - | |
| 8 | NFDM + Casein | 35 + 3.5 |
| 9 | NFDM + Casein | 35 + 3.5 |
| 10 | NFDM + Casein | 35 + 3.5 |
| 11 | - | |
| 12 | - | |
| 13 | - | |
| 14 | - | |
| 15 | - | |

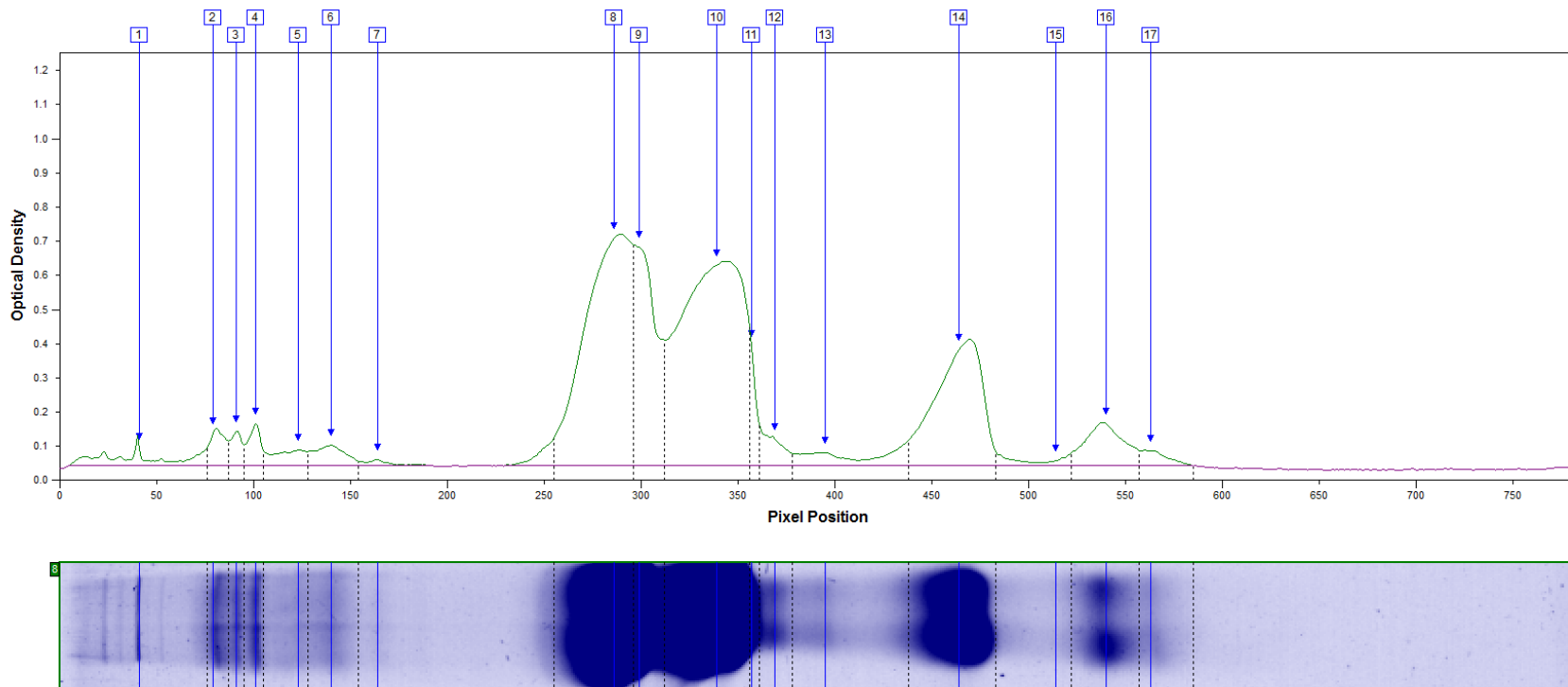Table 2. Key to loading of gel LE p.106#1.

4

Gel LE p.106 #1     lane 4          Sample: NFDM
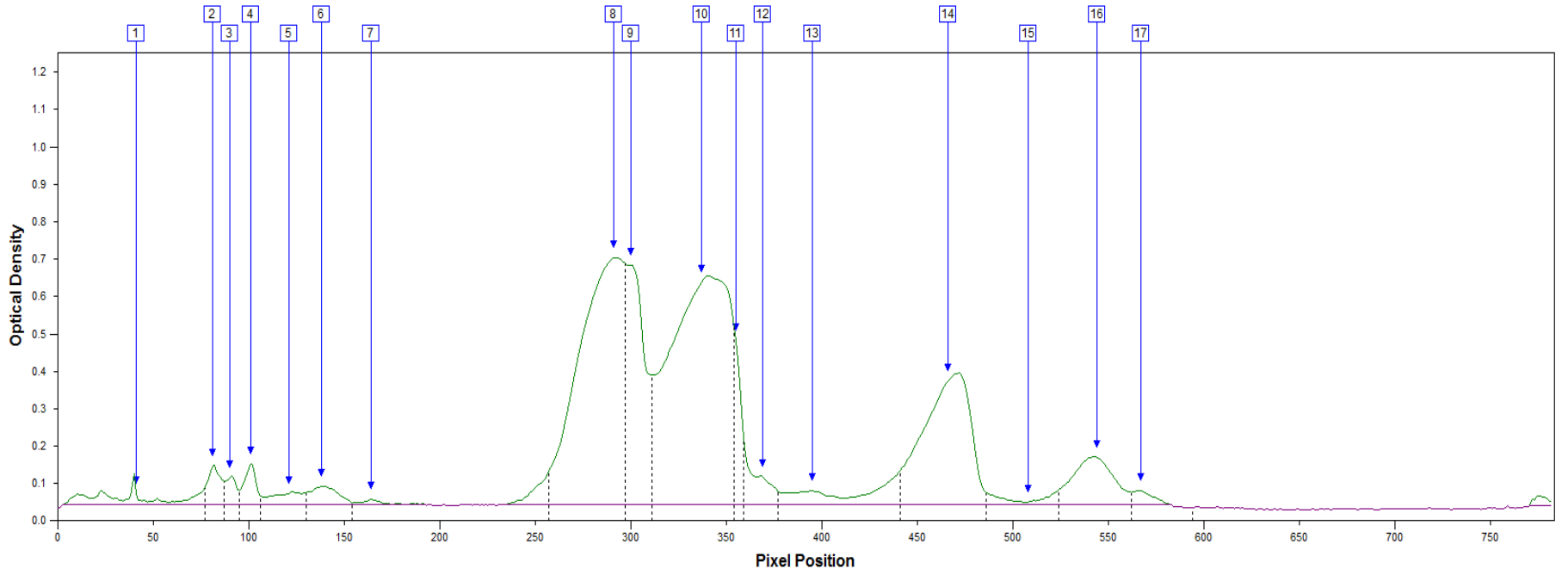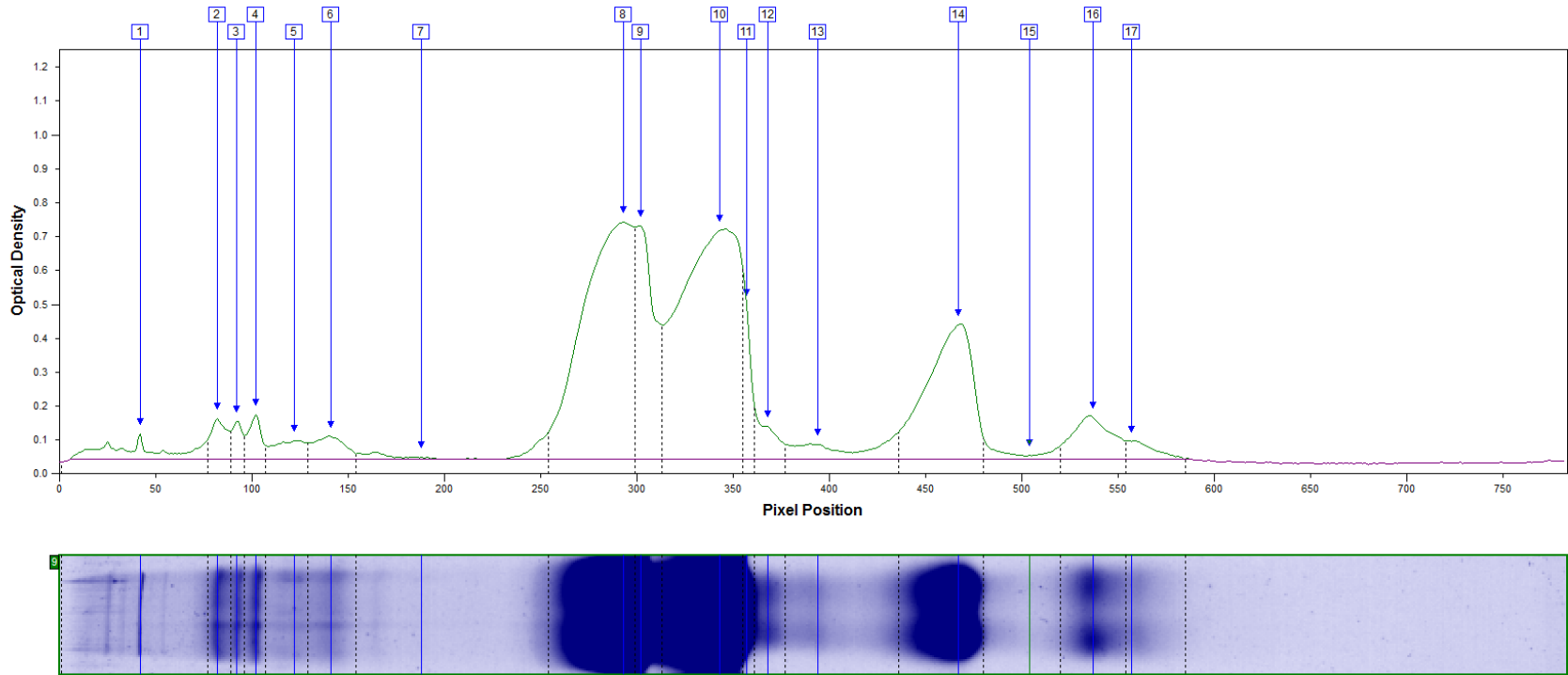
Gel LE p.106 #1     lane 5          Sample: NFDM
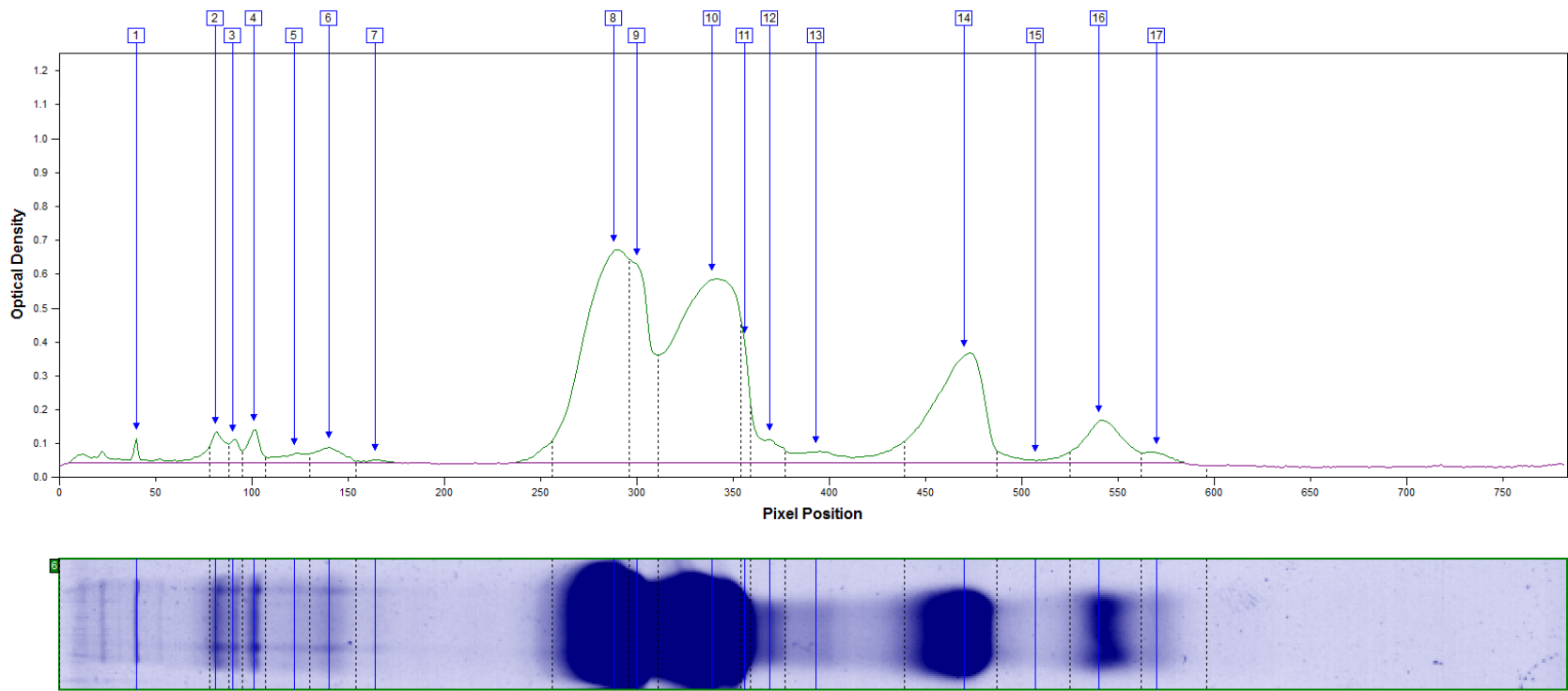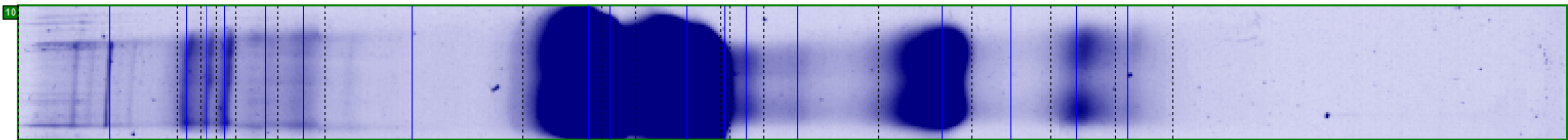
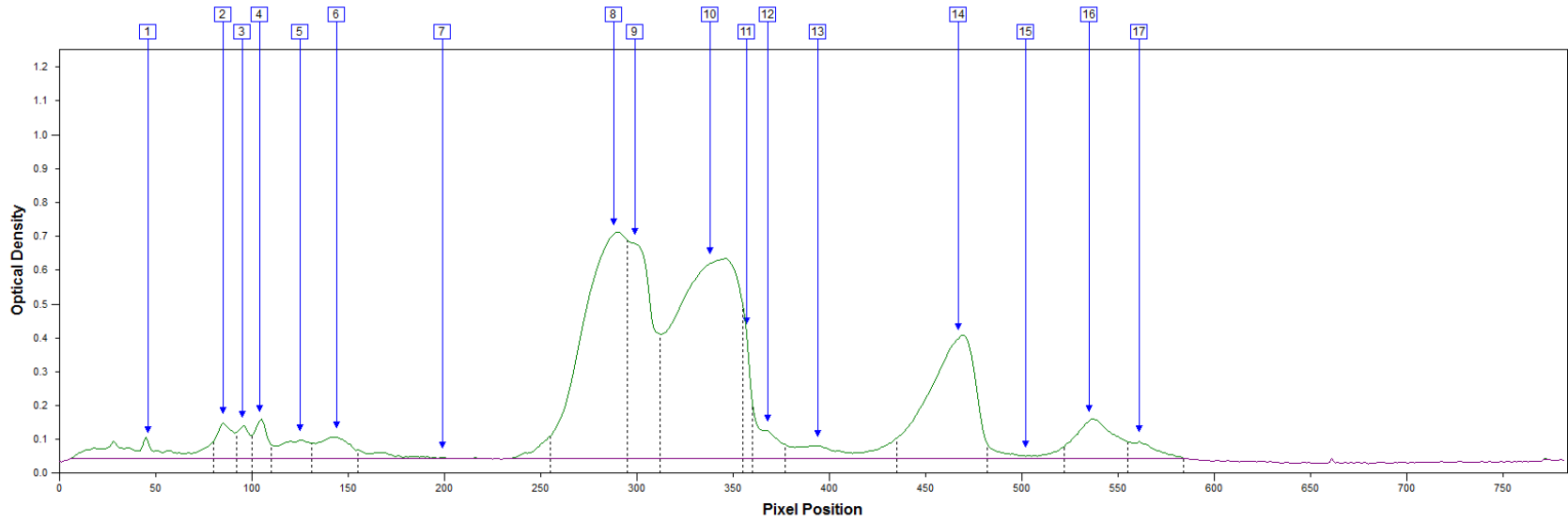Gel LE p.106 #1      lane 6          Sample: NFDM

Gel LE p.106 #1     lane 8          Sample: NFDM + Casein

Gel LE p.106 #1     lane 9          Sample: NFDM + Casein

Gel LE p.106 #1     lane 10          Sample: NFDM + Casein