

March 1, 2017

Client
Biology, Inc.
1000 Main St
Madison, WI 00000

Dear Ms. Client:

Results of the electrophoretic analysis of your soy protein samples, received 2/28/17, are presented on the following pages. The molecular weight (MW) range examined extended from ~2.5 to 100 kDa as shown in the enclosed peak density profile versus MW graphs. Table 1 shows the percentage of total stain per lane between the molecular weight ranges of >100 kDa, 50-100 kDa, 20-50 kDa, 10-20 kDa, and <10 kDa. Figure 1 shows the MW ranges marked on the gel. Figure 2 is an image of the dried Coomassie blue stained gel, Table 2 describes the gel loading scheme. Figures 3-11 show peak density profiles for the samples. The original dried gel and methods are also included.

Methods

The samples were dissolved in sample buffer containing 2.3% sodium dodecyl sulfate (SDS), 10% glycerol, 50 mM dithiothreitol, and 63 mM tris, pH 6.8 to bring the samples to a protein concentration of 20mg/ml. Following buffer addition, the samples were heated in a digital dry bath at 95° for 5 minutes. After sample cooled, the sample was diluted 2:1 with 6M HCl, and then heated at 60°, and 80° for 5 minutes and 10 minutes, and one aliquot heated at 95° for 40 minutes. The samples were cooled to room temperature and 5% NaOH was added to neutralize the HCl.

SDS slab gel electrophoresis was carried out using a 16.5% acrylamide peptide slab gel (Shagger, H. and Jagow, G. *Anal. Biochem.* 166:368, 1987) (0.75 mm thick). Electrophoresis was performed using 15mAmp/gel overnight at which time the bromophenol blue front had migrated to the end of the slab gel. Following slab gel completion, the gel was stained with Coomassie blue dye, destained in 10% acetic acid until a clear background was obtained, and dried between cellophane sheets.

The following proteins (Sigma Chemical Co., St. Louis, MO and EMD Millipore, Billerica, MA) were added as molecular weight standards: phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), myoglobin from horse heart (17,000), lysozyme (14,000), aprotonin from bovine lung (6,500), and insulin chain b, oxidized, bovine (3,496).

The stained gel was digitized over the appropriate optical density range using a calibrated GE Healthcare Image Scanner III. Molecular weights were calculated from the molecular weight standards using Phoretix 1D software (version 11.2) with a Windows 10 compatible computer and a first order lagrange molecular weight curve.

Please do not hesitate to call with any questions or comments.

Sincerely,

Lindsey Eierman
Biochemist

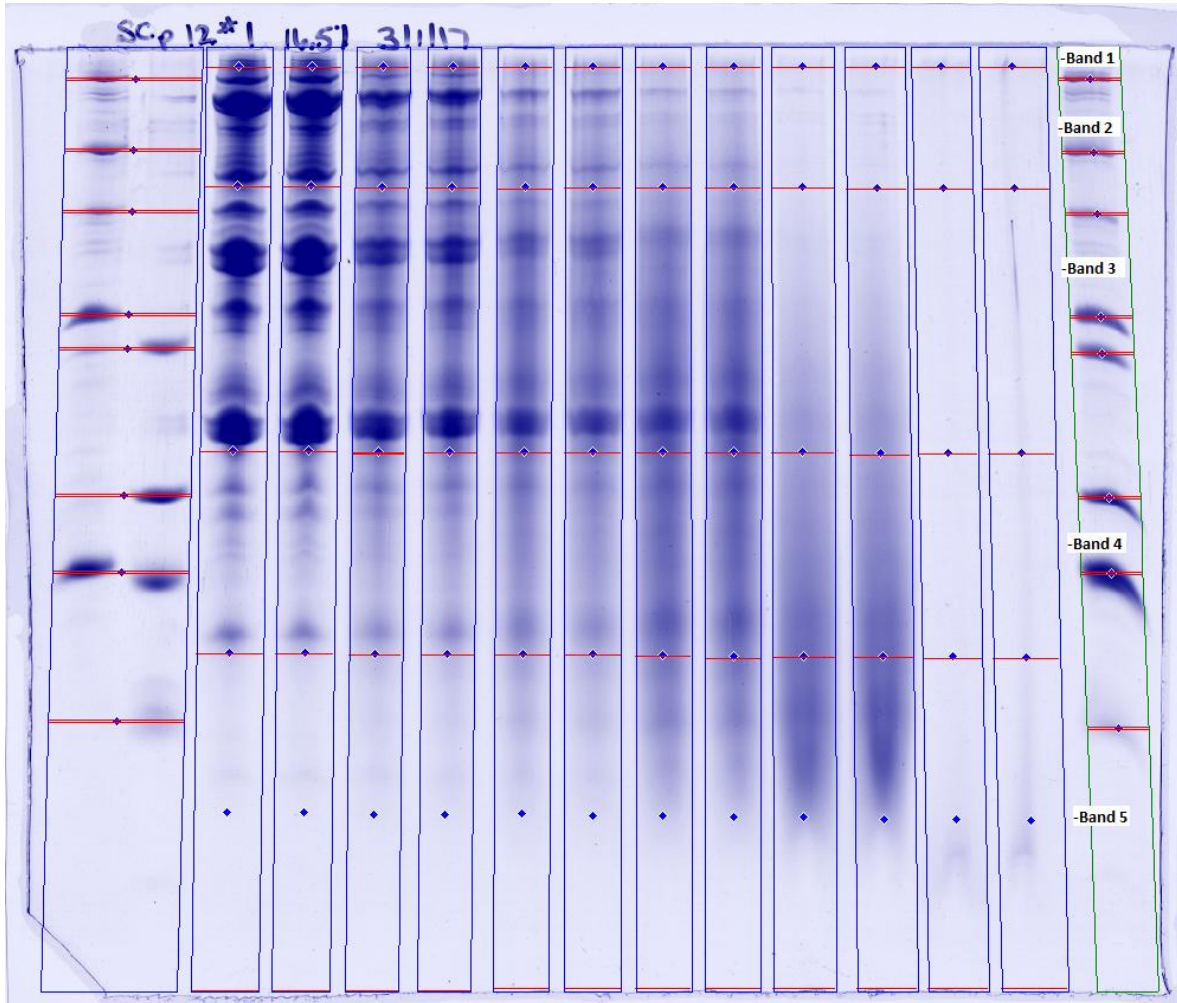


Figure 1. Image of band marking (MW ranges) for gel SC p.12#1.

Mol. Wt. Range	Band	No Heat, No HCl		Heat 5 min at 60°C		Heat 10 min at 60°C		Heat 5 min at 80°C		Heat 10 min at 80°C		Heat 40 min at 95°C	
		Ave.	±SD	Ave.	±SD	Ave.	±SD	Ave.	±SD	Ave.	±SD	Ave.	±SD
>100 kDa	1	2.91	0.22	2.04	0.10	0.92	0.10	0.38	0.01	0.13	0.02	14.18	3.60
50-100 kDa	2	32.42	0.23	24.18	0.23	14.72	0.10	7.45	0.13	0.81	0.18	13.10	6.56
20-50 kDa	3	53.47	0.08	54.06	0.18	52.30	0.49	44.34	0.00	19.14	0.09	0.36	0.50
10-20 kDa	4	10.80	0.02	17.10	0.10	25.17	0.24	33.25	0.25	40.82	0.21	0.00	0.00
<10 kDa	5	0.41	0.07	2.62	0.25	6.88	0.05	14.58	0.12	39.11	0.08	72.37	10.65

Table 1. Percent of total protein in the sample relative to total stain density between the molecular weight ranges of >100 kDa, 50-100 kDa, 20-50 kDa, 10-20 kDa, and <10 kDa for gel SC p.12 #1.

Mol. Wt. Range	Band	No Heat, No HCl		Heat 5 min at 60°C		Heat 10 min at 60°C		Heat 5 min at 80°C		Heat 10 min at 80°C		Heat 40 min at 95°C	
		Ave.	±SD	Ave.	±SD	Ave.	±SD	Ave.	±SD	Ave.	±SD	Ave.	±SD
>100 kDa	1	205.39	17.66	126.47	7.24	51.11	6.94	26.84	0.81	6.37	1.09	8.95	0.88
50-100 kDa	2	2290.80	7.11	1497.66	26.12	814.13	25.28	519.57	16.07	41.16	8.99	7.90	1.32
20-50 kDa	3	3778.87	33.11	3348.89	14.48	2890.97	44.00	3091.18	41.17	969.73	3.18	0.18	0.25
10-20 kDa	4	762.89	9.42	1048.99	0.20	1391.92	47.16	2317.84	13.69	2068.23	26.66	0.00	0.00
<10 kDa	5	29.02	4.92	167.23	7.01	380.60	12.16	1016.85	21.79	1981.48	19.57	49.02	23.66

Table 2. Total stain volume between the molecular weight ranges of >100 kDa, 50-100 kDa, 20-50 kDa, 10-20 kDa, and <10 kDa for gel SCp.12#1.

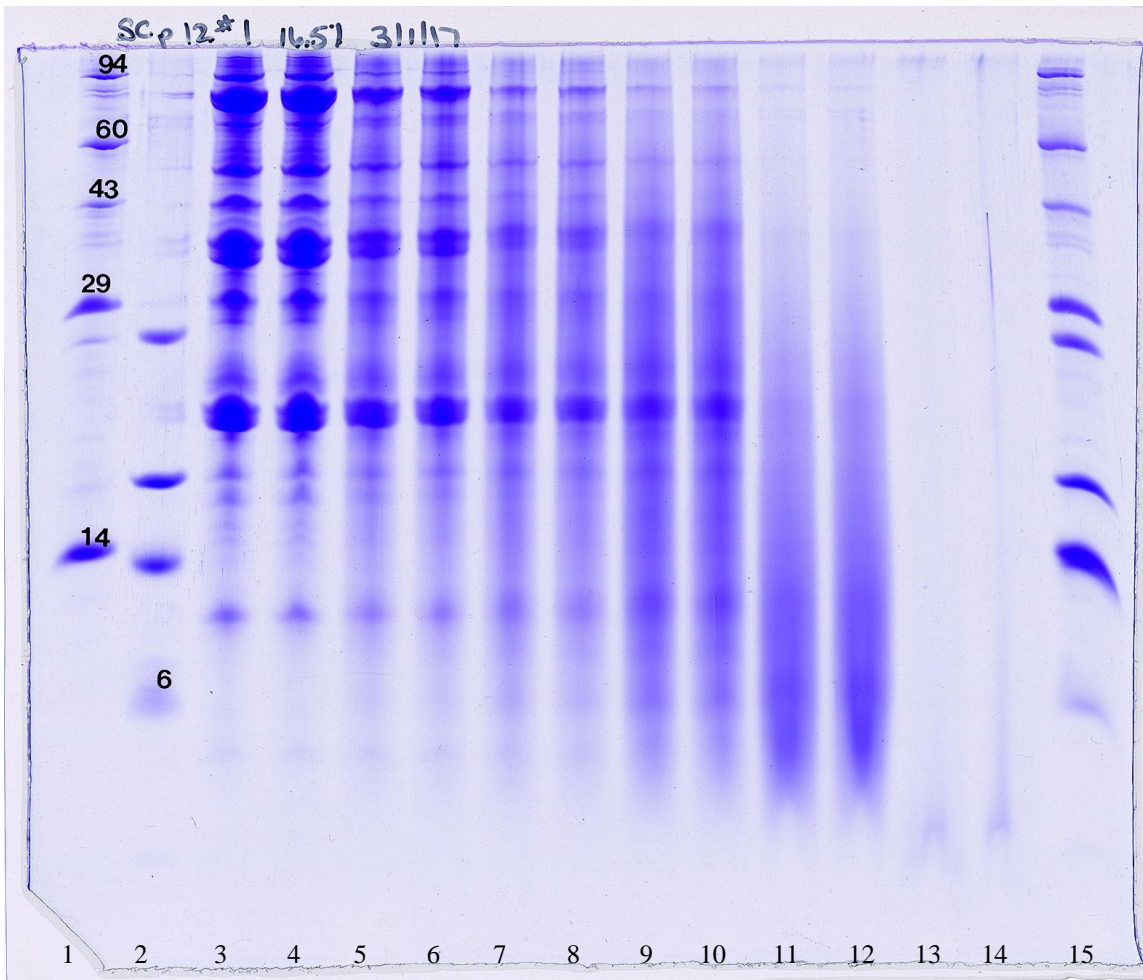


Figure 2. Image of gel SC p.12#1.

Lane	Sample	$\mu\text{g Protein}$	$\mu\text{g Sample}$	$\mu\text{l Loaded}$
1	High Range Molecular Weight Standards	-	-	5
2	Low Range Molecular Weight Standards	-	-	15
3	No heat, No HCl	50	89.41	4.13
4	No heat, No HCl	50	89.41	4.13
5	Heat 5min, at 60°C	50	89.41	4.13
6	Heat 5min, at 60°C	50	89.41	4.13
7	Heat 10min, at 60°C	50	89.41	4.13
8	Heat 10min, at 60°C	50	89.41	4.13
9	Heat 5min, at 80°C	50	89.41	4.13
10	Heat 5min, at 80°C	50	89.41	4.13
11	Heat 10min, at 80°C	50	89.41	4.13
12	Heat 10min, at 80°C	50	89.41	4.13
13	Heat 40min, at 95°C	50	89.41	9.14
14	Heat 40min, at 95°C	50	89.41	9.14
15	High and Low Range Molecular Weight Standards	-	-	5/15

Table 3. Key to loading gel SC p.12#1.

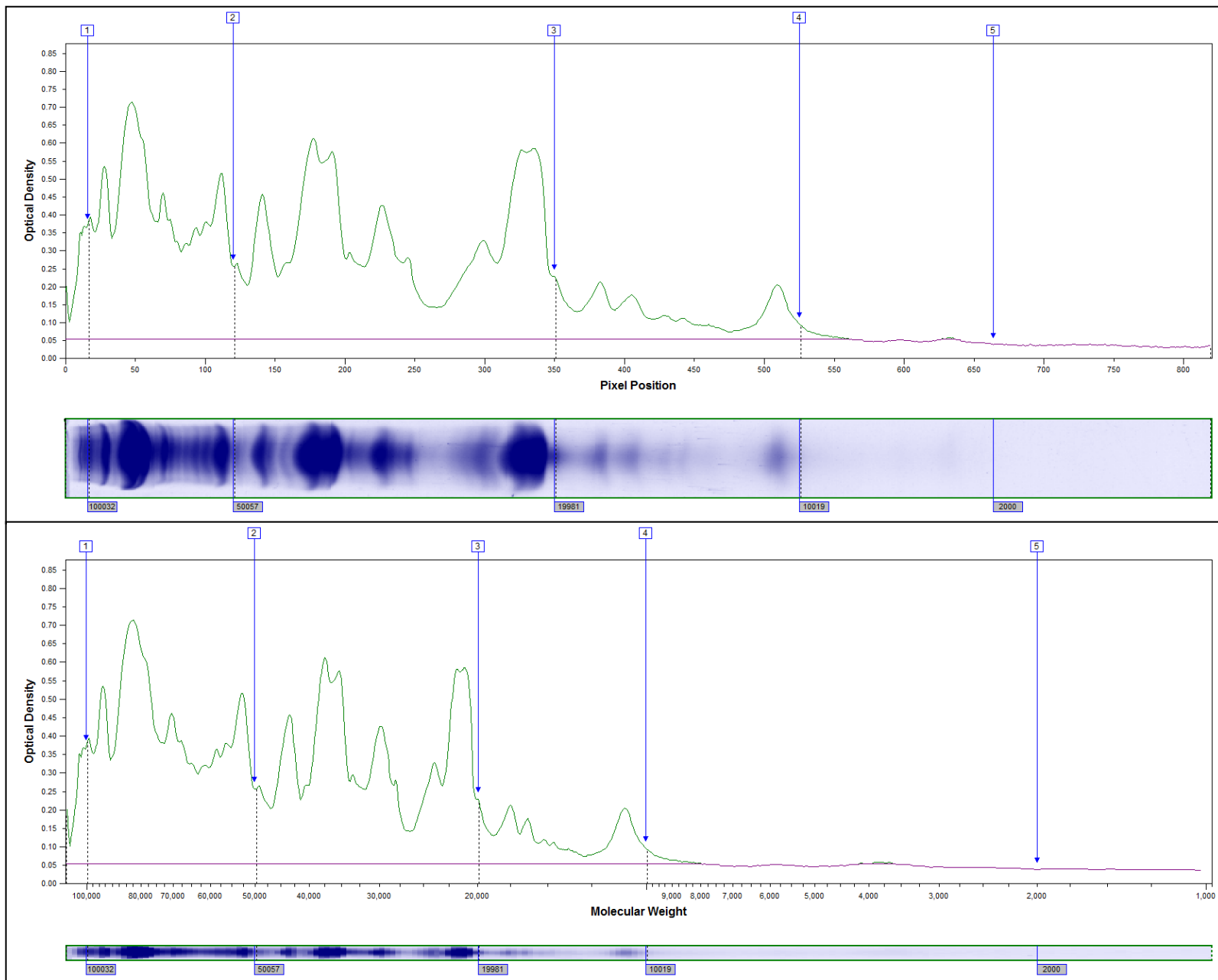


Figure 3a and b. Gel SC p.12#1, Lane 3. Sample: No Heat, No HCl. Flags under lane indicate calibrated molecular weight. Figure 3a is the lane histogram based on pixel position, and Figure 3b is the lane histogram based on calibrated molecular weight.

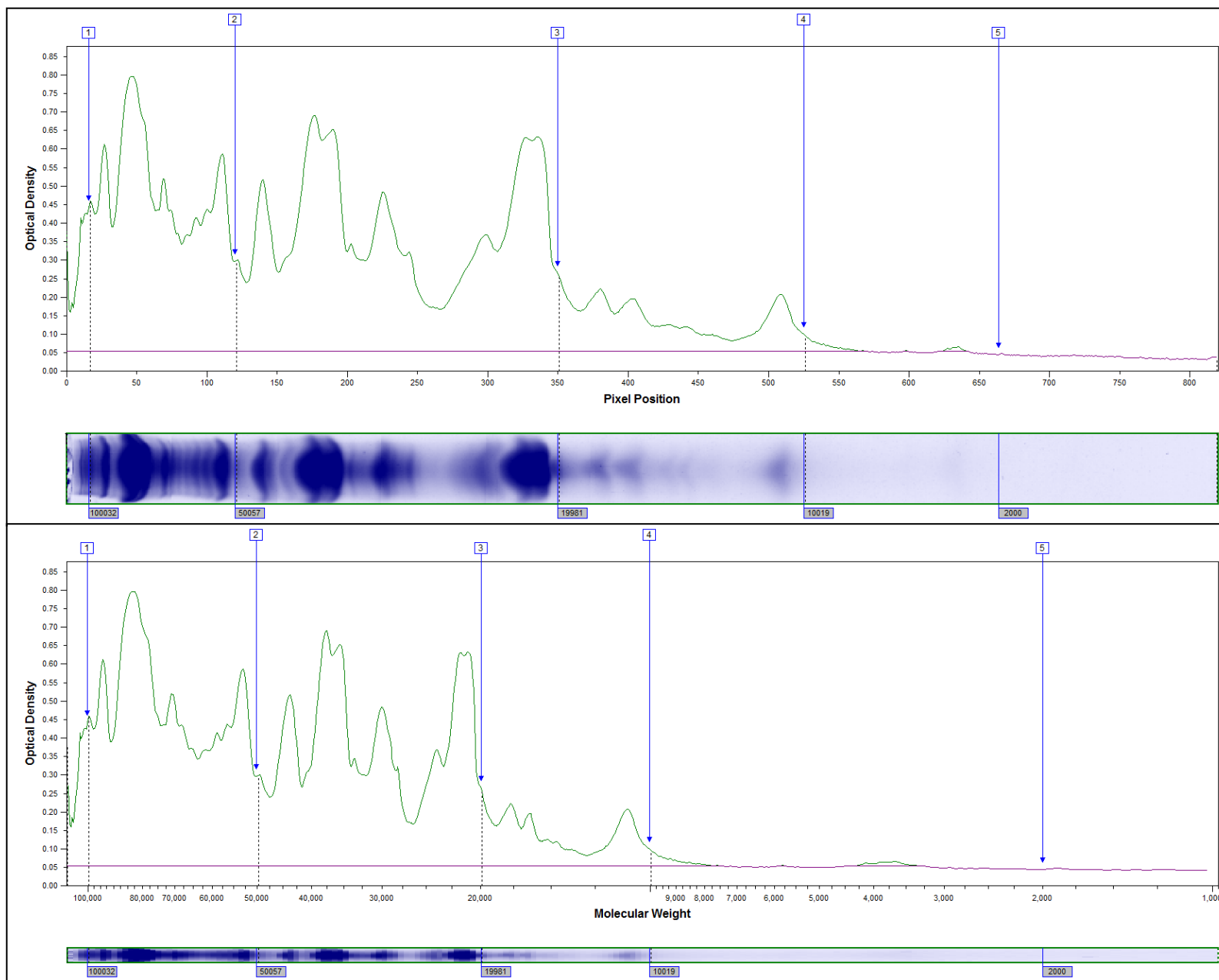
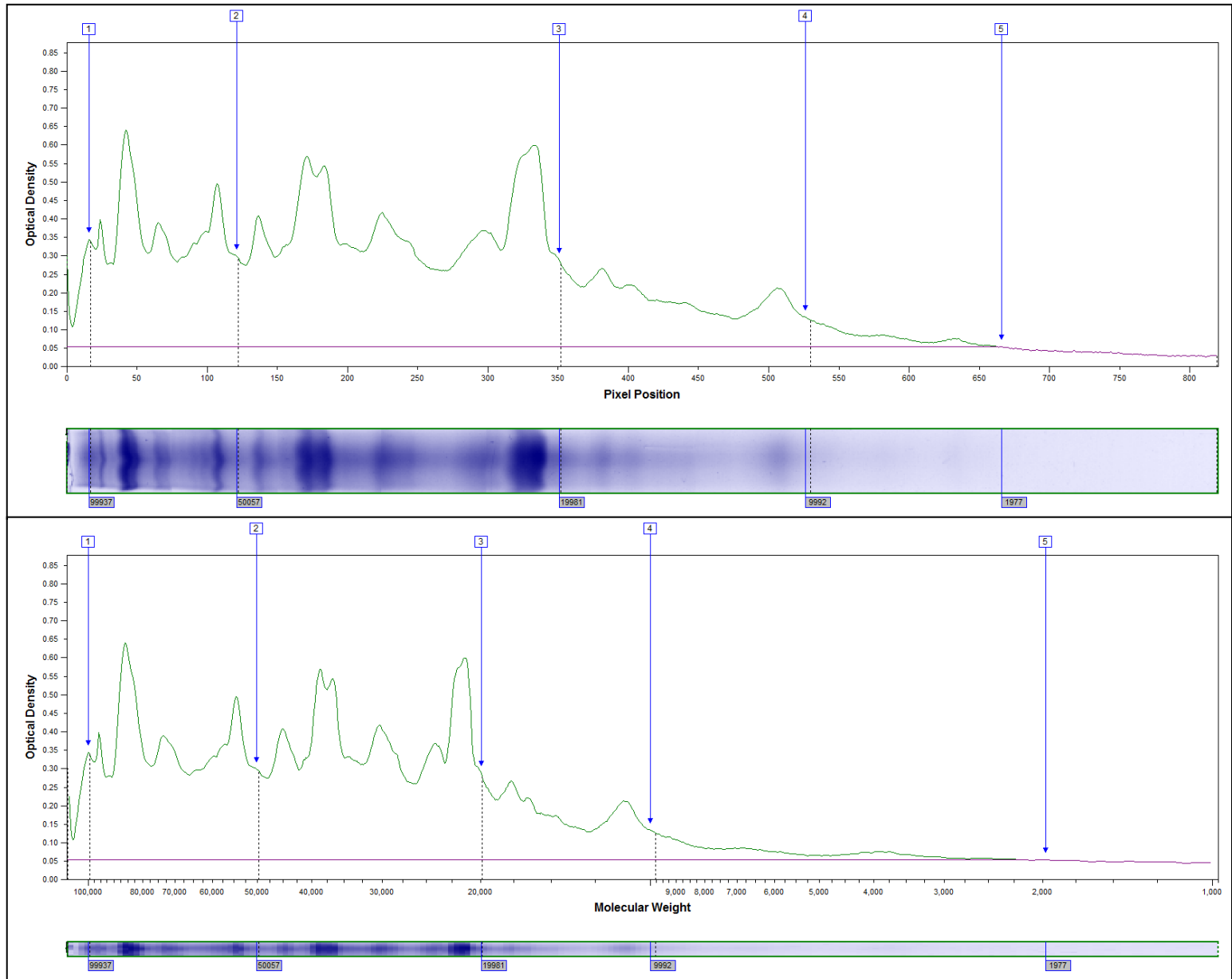


Figure 4a and b. Gel SC p.12#1, Lane 4. Sample: No Heat, No HCl. Flags under lane indicate calibrated molecular weight. Figure 4a is the lane histogram based on pixel position, and Figure 4b is the lane histogram based on calibrated molecular weight.



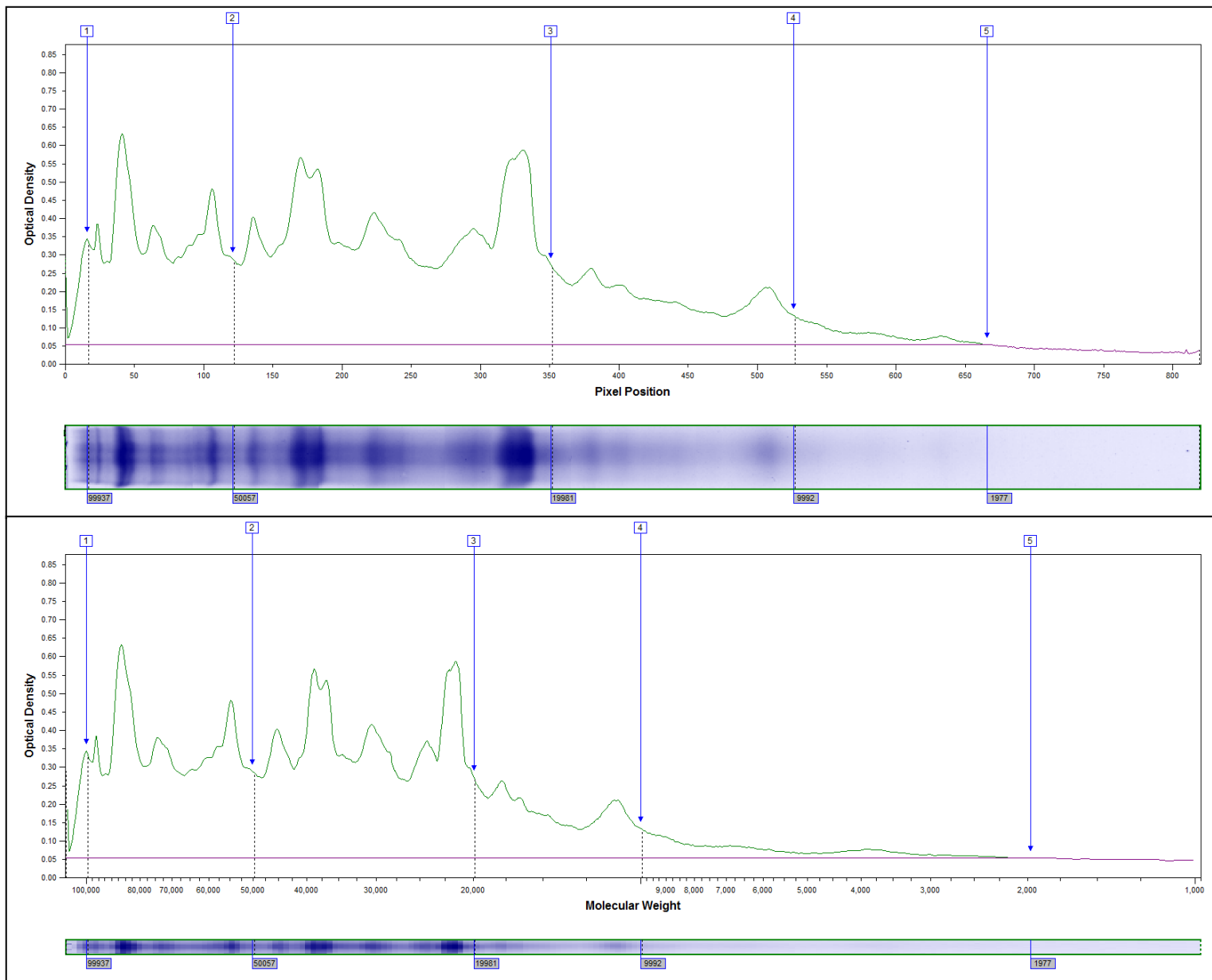


Figure 6a and b. Gel SC p.12#1, Lane 6. Sample: Heat 5 minutes at 60°. Flags under lane indicate calibrated molecular weight. Figure 6a is the lane histogram based on pixel position, and Figure 6b is the lane histogram based on calibrated molecular weight.

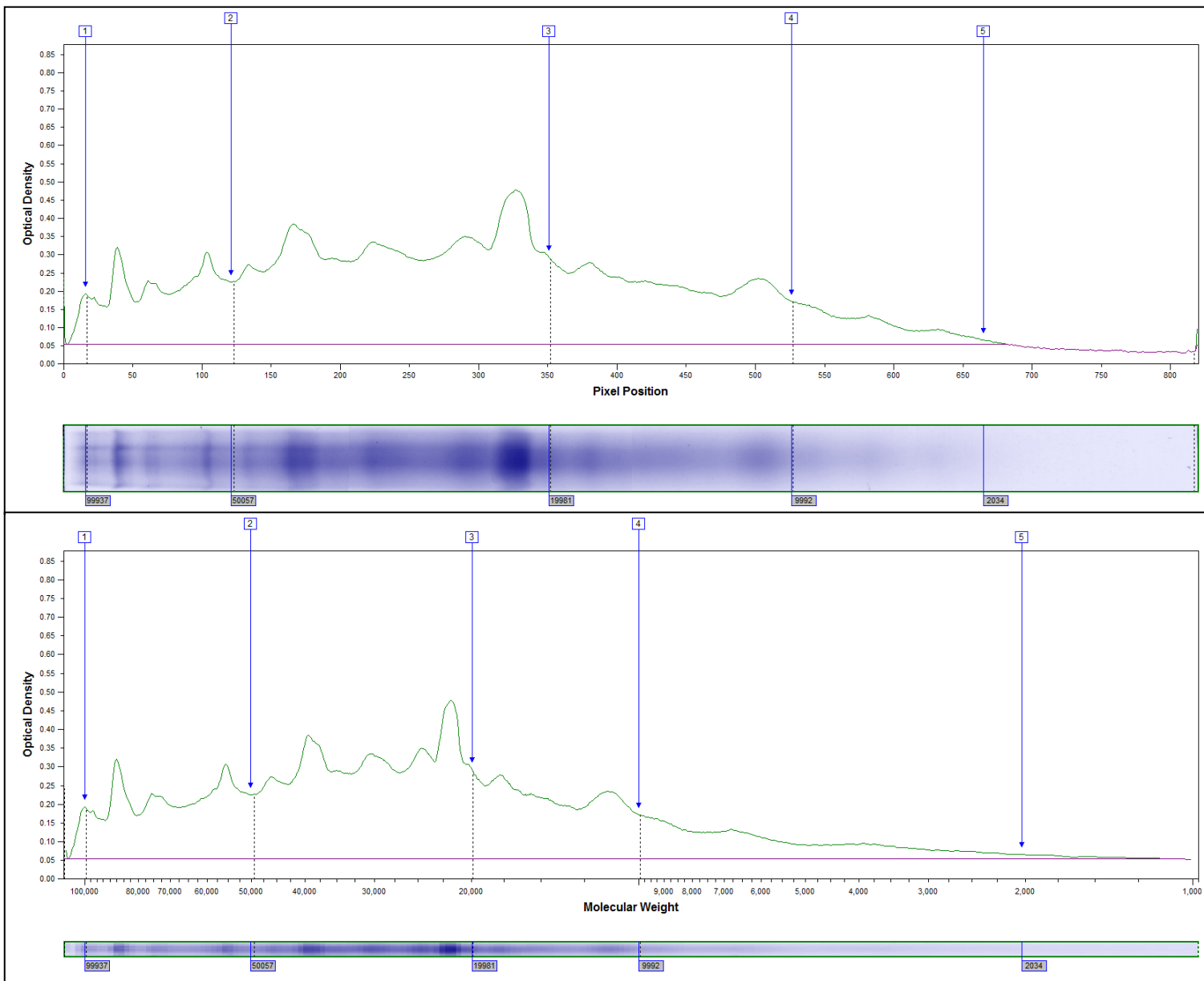


Figure 7a and b. Gel SC p.12#1, Lane 7. Sample: Heat 10 minutes at 60°C. Flags under lane indicate calibrated molecular weight. Figure 7a is the lane histogram based on pixel position, and Figure 7b is the lane histogram based on calibrated molecular weight.

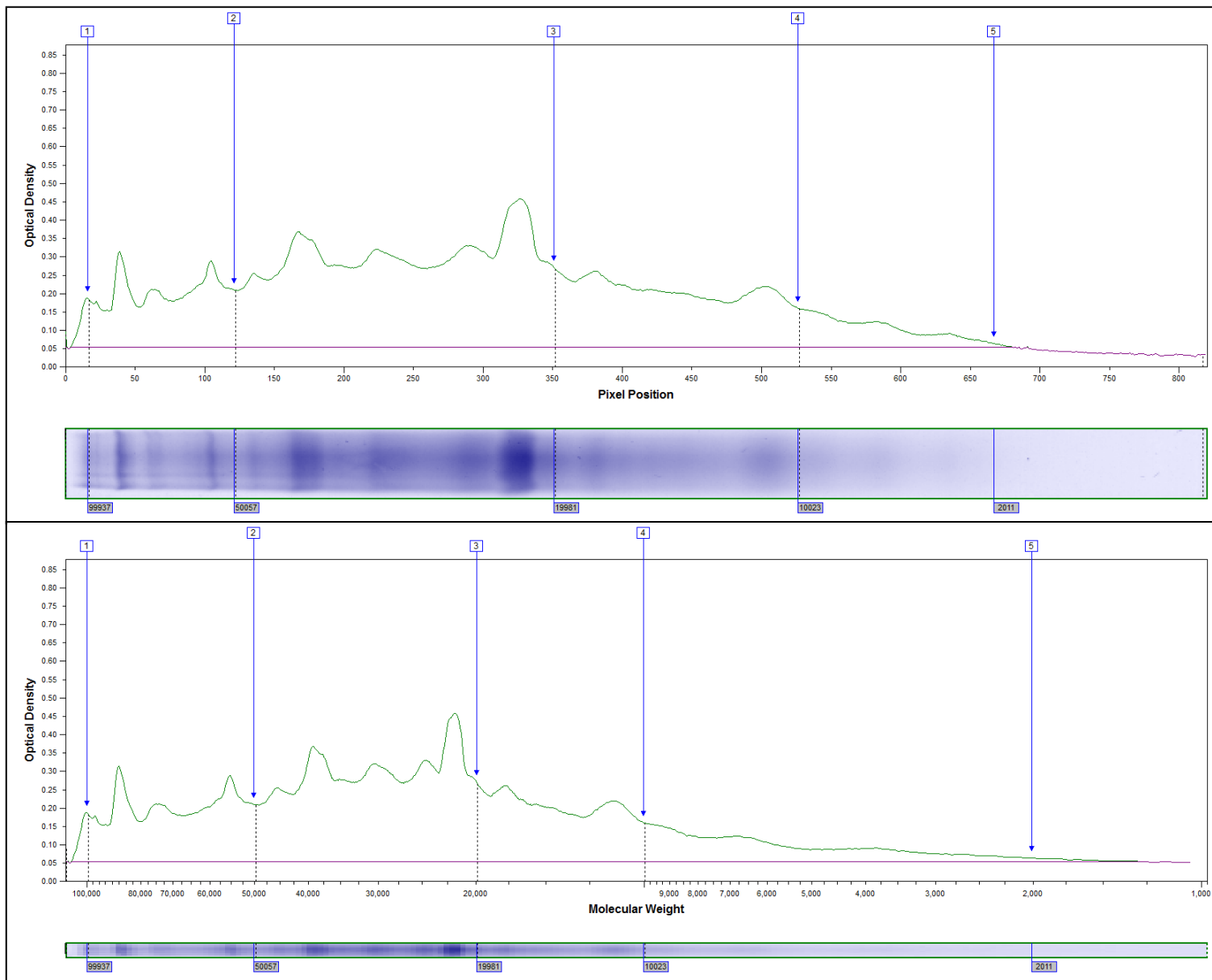


Figure 8a and b. Gel SC p.12#1, Lane 8. Sample: Heat 10 minutes at 60°C. Flags under lane indicate calibrated molecular weight. Figure 8a is the lane histogram based on pixel position, and Figure 8b is the lane histogram based on calibrated molecular weight.

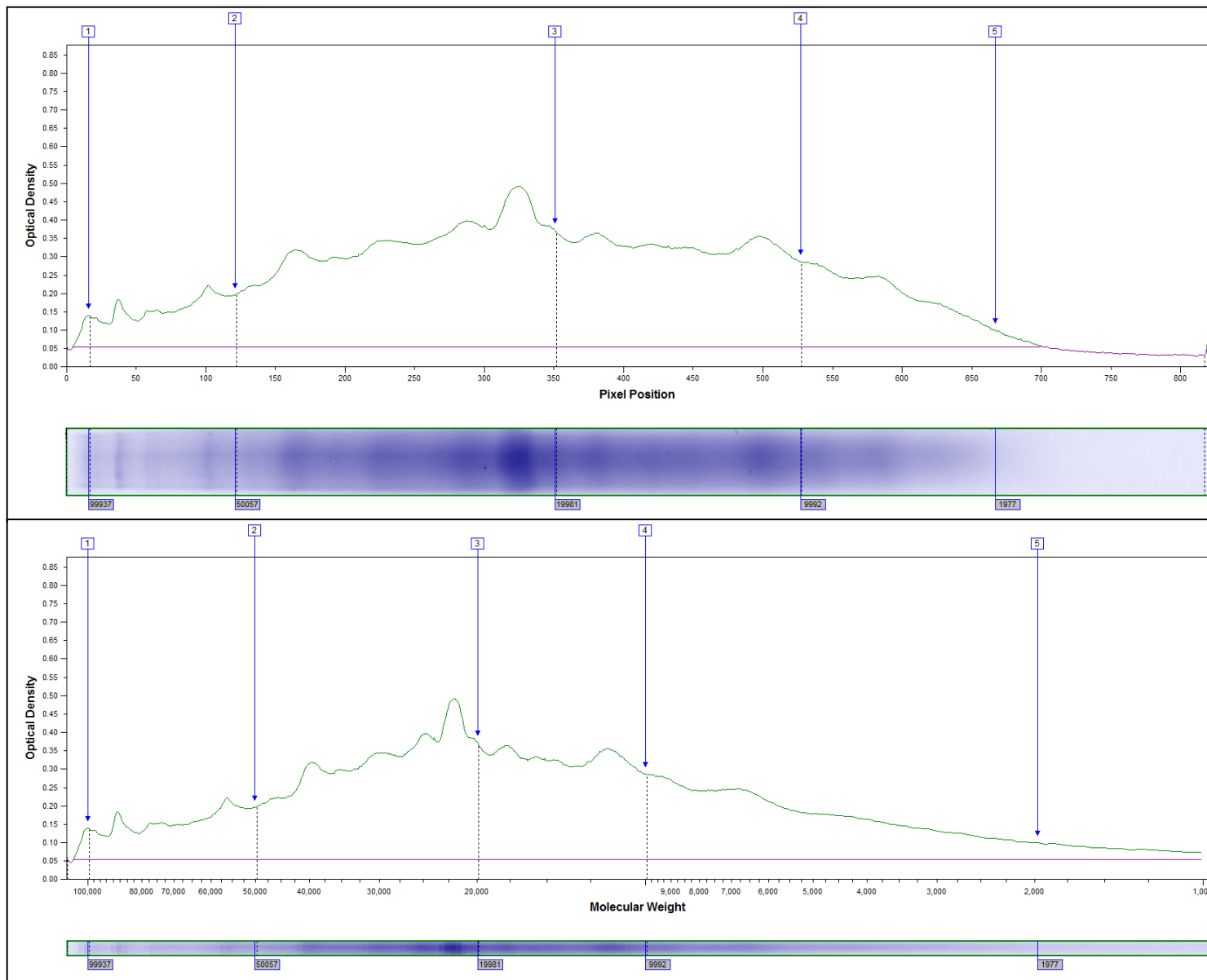


Figure 9a and b. Gel SC p.12#1, Lane 9. Sample: Heat 5 minutes at 80°C. Flags under lane indicate calibrated molecular weight. Figure 9a is the lane histogram based on pixel position, and Figure 9b is the lane histogram based on calibrated molecular weight.

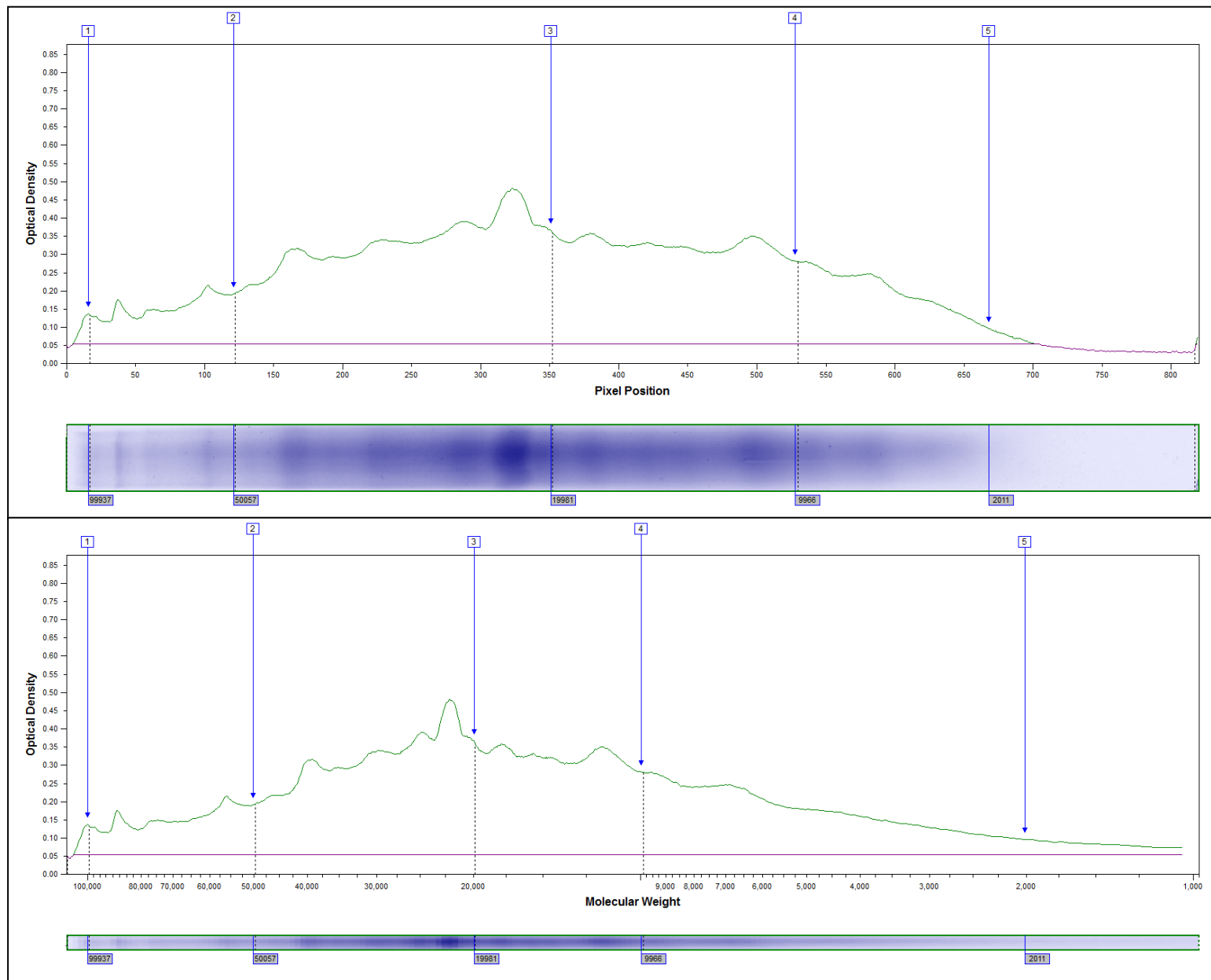


Figure 10a and b. Gel SC p.12#1, Lane 10. Sample: Heat 5 minutes at 80°C. Flags under lane indicate calibrated molecular weight. Figure 10a is the lane histogram based on pixel position, and Figure 10b is the lane histogram based on calibrated molecular weight.

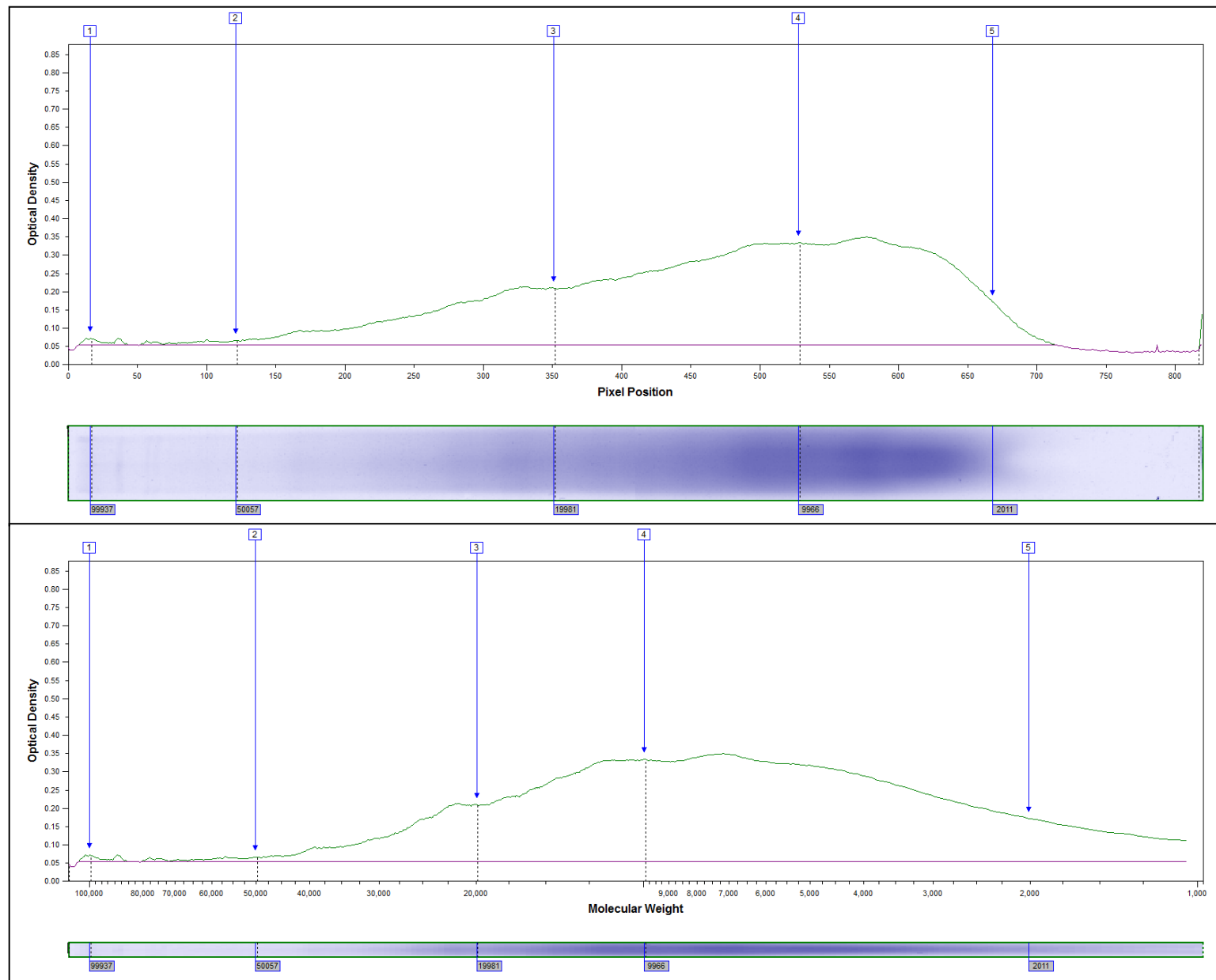


Figure 11a and b. Gel SC p.12#1, Lane 11. Sample: Heat 10 minutes at 80°C. Flags under lane indicate calibrated molecular weight. Figure 11a is the lane histogram based on pixel position, and Figure 11b is the lane histogram based on calibrated molecular weight.

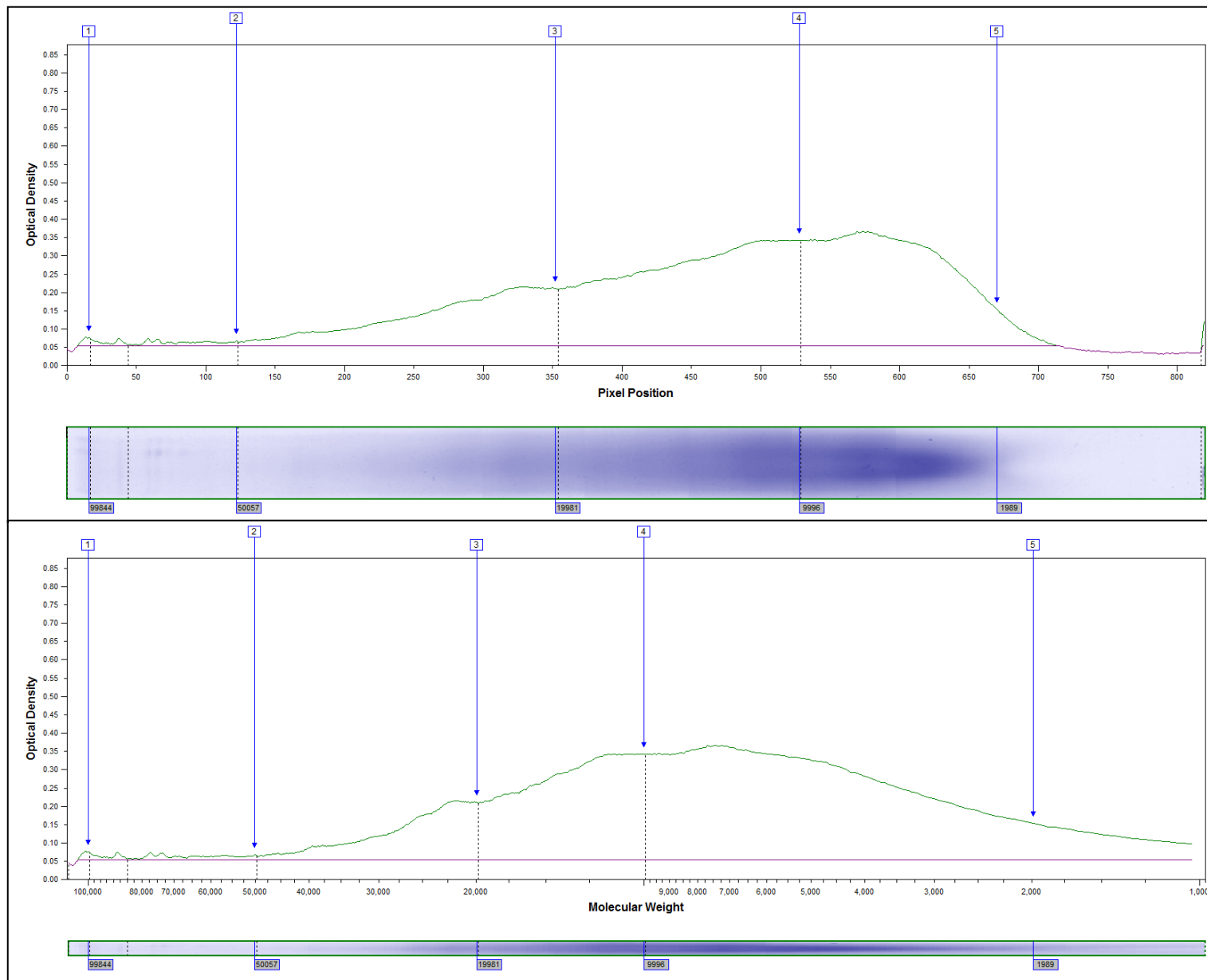


Figure 12a and b. Gel SC p.12#1, Lane 12. Sample: Heat 10 minutes at 80°C. Flags under lane indicate calibrated molecular weight. Figure 12a is the lane histogram based on pixel position, and Figure 12b is the lane histogram based on calibrated molecular weight.

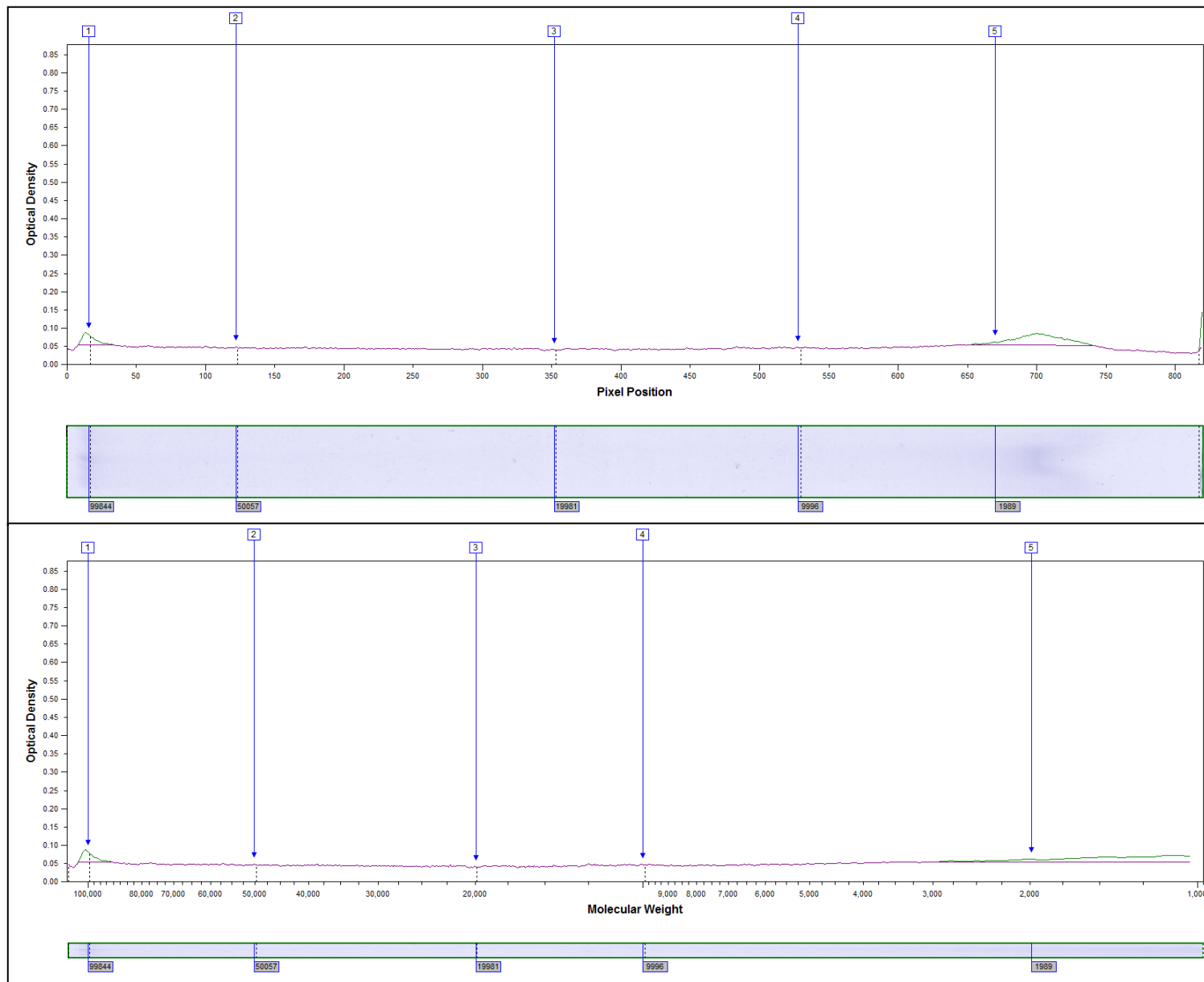


Figure 13a and b. Gel SC p.12#1, Lane 13. Sample: Heat 40 minutes at 95°C. Flags under lane indicate calibrated molecular weight. Figure 13a is the lane histogram based on pixel position, and Figure 13b is the lane histogram based on calibrated molecular weight.

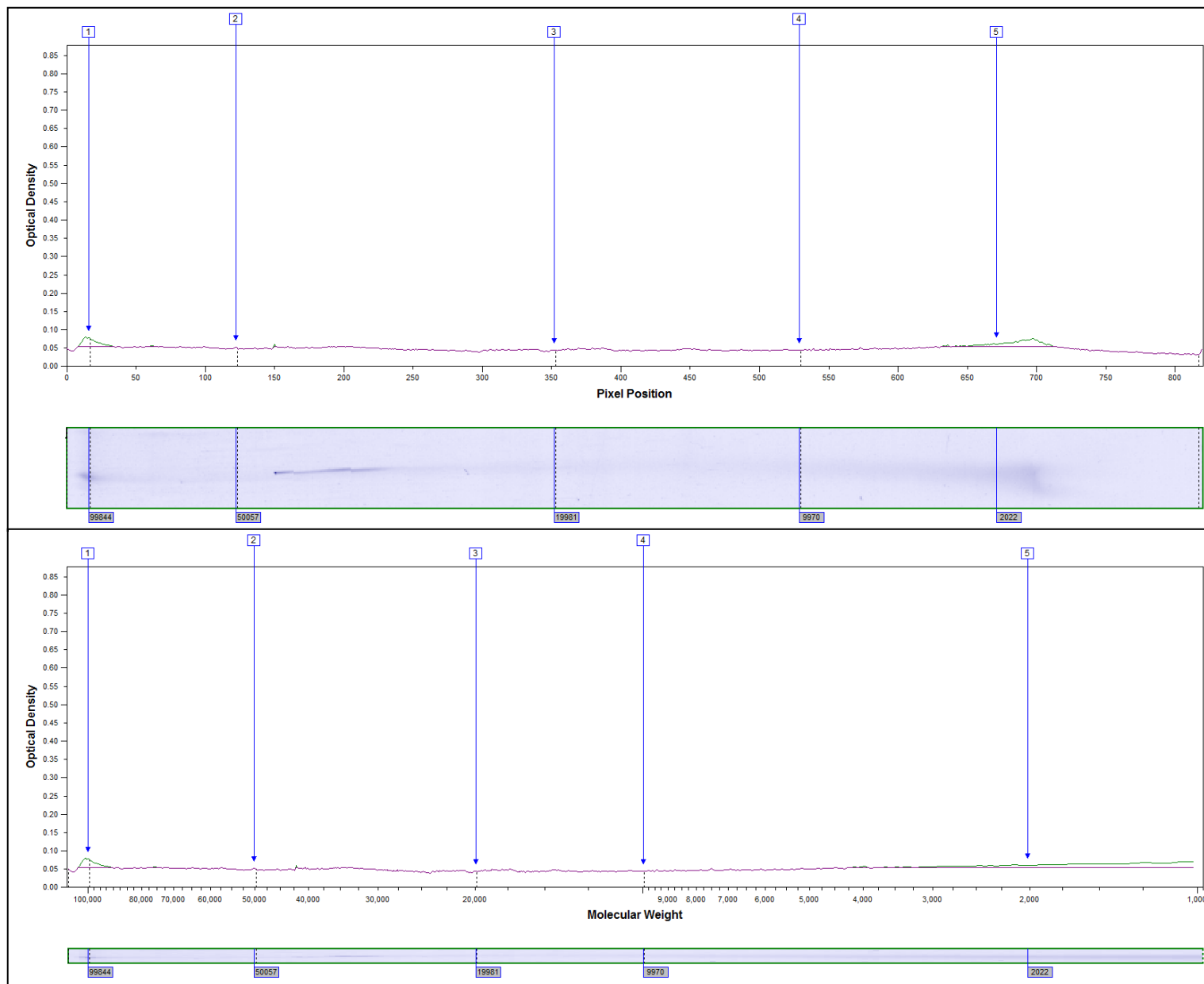


Figure 14a and b. Gel SC p.12#1, Lane 14. Sample: Heat 40 minutes at 95°C. Flags under lane indicate calibrated molecular weight. Figure 14a is the lane histogram based on pixel position, and Figure 14b is the lane histogram based on calibrated molecular weight.