# Protein SDS Gel Electrophoresis

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**Tradename:** ACB Botanical Sugar Complex

**Code:** 20039

**CAS #:** 9005-25-8 & 68333-16-4

**Test Request Form #:** 4453

**Lot #:** 59321

**Sponsor:** *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
**Study Director:** *Maureen Danaher*
**Principal Investigator:** *Jennifer Goodman*

**Test Performed:**
Protein SDS Gel Electrophoresis

## Introduction

Molecular weight is a critical component of topical cosmetic products given compounds with larger molecular weights do not penetrate the skin as deep as smaller molecular weight compounds. Molecular weights can also be utilized to determine the impact of cosmetic products on specific molecules of interest. Specifically, the relative amount of a molecule within a given range can be assessed, providing insight to an increase or decrease in particular molecules that impact the overall function of skin differently.

Accordingly, Protein SDS Gel Electrophoresis was performed to provide a visualization of molecular weight for **ACB Botanical Sugar Complex**.

## Assay Principle

In vertical gel electrophoresis the wells are loaded in a vertical position and as the current runs through the gel the bands, visible due to the loading dye, move through the gel top to bottom. As the current increases, the speed at which the bands move through the gel increases. This can lead to band warping and lack of distinct band formation. Due to this, moderate currents with longer run times are generally chosen for better downstream results. When samples are run with known standard molecular weight ladders, gel electrophoresis becomes a beneficial tool for determining molecular weight of a sample. Running protein samples alongside one another also demonstrates a visual comparison of band migration and intensity sample to sample. Protein gel electrophoresis provides both quantitative as well as qualitative data for protein samples of interest.

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## Materials

| | |
|---|---|
| **A. Run Conditions:** | 90 Volts for 60 minutes |
| **B. Equipment:** | Mini Gel Tank (Invitrogen)*; Power Supply (Hoefer)*; Pipettes |
| **C. Gel:** | 12-well 10% Polyacrylamide |
| **D. Reagents:** | SureCast Acrylamide 40%; SureCast Resolving Buffer; Distilled Water; 10% SureCast APS; SureCast TEMED; Simply Blue SafeStain; NuPAGE SDS Running Buffer; Native Tris-Glycine Sample Buffer (2X) |
| **E. Other:** | Spectra Multicolor Broad Range Protein Ladder 260-10 kDa (Thermo Scientific)*; Spectra Multicolor Low Range Protein Ladder 42-1.7 kDa (Thermo Scientific)* |

*Or suitable alternatives, subject to change without notice based off vendor availability

## Methods

The SureCast plate and gel casting system was used to prepare the polyacrylamide gels. The gels were prepared using the specifications listed in the SureCast system guidelines specific to the particular grade gel chosen for the sample type. A 12-well comb was used to create wells for up to 12 samples to be loaded. Once the gels had hardened, the comb was removed, and the gels were rinsed twice with 1X NuPAGE SDS Running Buffer. The casting plates were locked into the mini gel tank and the remaining running buffer was used to fill the tank. The samples were diluted in a 1:8 sample:loading buffer ratio and 15 µL of each sample mixture were added to the designated wells. 15 µL of the Spectra Multicolor Low Range Protein Ladder was added to the designated well.

The gel was run for 60 minutes at 90V, removed from the glass casting plates, and rinsed with distilled water three times for five minutes each rinse with slight agitation. The gel was then fully submerged in the Simply Blue SafeStain for 2.5 hours with intermittent agitation and then rinsed in distilled water for up to one hour. Digital images of the gel were taken after the water rinse.

## Results

The data obtained from this study met criteria for a valid assay and the protein ladder performed as anticipated.

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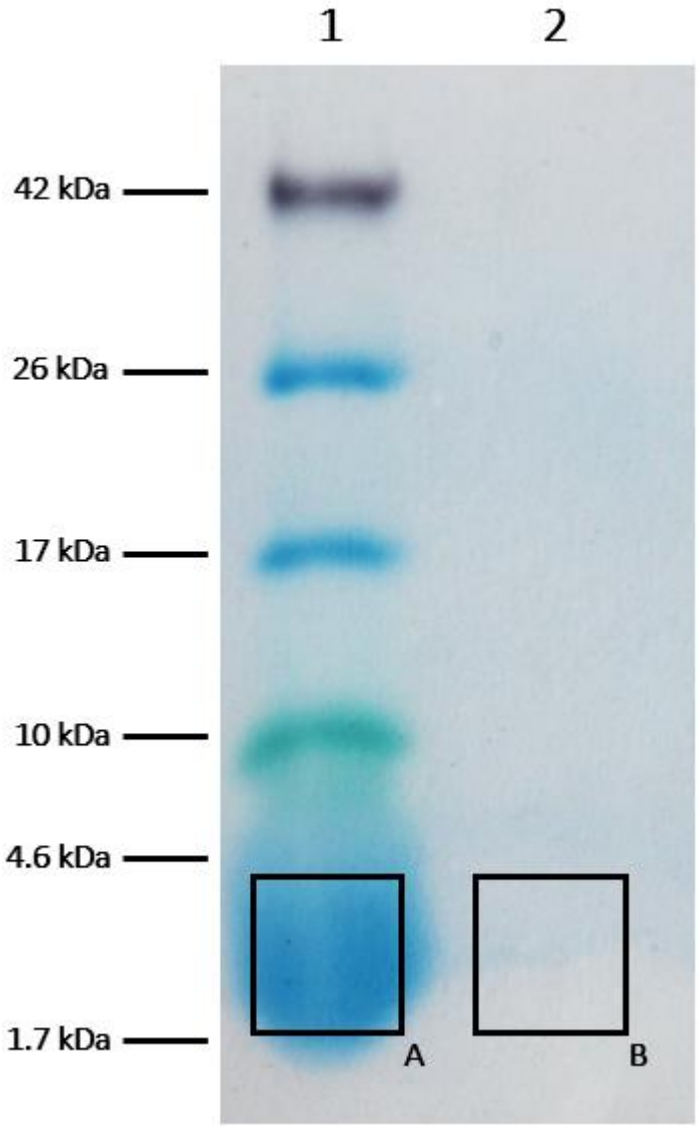


**Figure 1.** Final Stained Gel Comparing **ACB Botanical Sugar Complex** to the Low Molecular Weight Protein Ladder.

**Table 1.** Mean Region of Interested Intensity Values of **ACB Botanical Sugar Complex.** High intensity is indicative of higher protein levels while lower intensities indicate a lack of protein within the outlined Regions of Interest.

| | Region of Interest A | Region of Interest B |
|---|---|---|
| Mean Intensity | 131.6 | 48.7 |

## Discussion

The migration rate of the bands directly correlates with the molecular weight of the sample material. The larger the molecular weight of a sample, the heavier the band is and the shorter distance it will travel down the gel. In contrast, the smaller the molecular weight of the sample, the lighter the band is and the further distance it will travel down the gel.

As shown in Figure 1, **ACB Botanical Sugar Complex** did not display detectable protein banding. This indicates a low molecular weight below 1.7 kDa, which has been suggested to allow easier interaction with the skin when compared to larger molecular weight materials.