Molecular Weight Estimation

Run the standards and samples on an SDS-PAGE gel. Process the gel with the desired stain and then destain to visualize the protein bands. Determine the R<sub>i</sub> graphically or using Quantity One® analysis software (or equivalent).

1. Using a ruler, measure the migration distance from the top of the resolving gel to each standard band and to the dye front.

2. For each band in the standards, calculate the R<sub>i</sub> value using the following equation:
   \[ R_i = \frac{\text{migration distance of the protein}}{\text{migration distance of the dye front}} \]

3. Repeat this step for the unknown bands in the samples.

4. Use a graphing program, plot the log (MW) as a function of R<sub>i</sub>.

5. Generate the equation \( y = mx + b \), and solve for \( y \) to determine the MW of the unknown protein.

Fig. 1. Example showing MW determination of an unknown protein.
Lane 1, 10 μl of Precision Plus Protein™ unstained standards; lanes 2–8, a dilution series of an *E. coli* lysate containing a hypothetical unknown protein (GFP). Proteins were separated by SDS-PAGE in a Criterion™ 4–20% Tris-HCl gel and stained with Bio-Safe™ Coomassie stain. Gel shown is the actual size.

\[
\begin{align*}
y &= -1.9944x + 2.7824 \\
r^2 &= 0.997
\end{align*}
\]

Fig. 2. Determining the MW of an unknown protein by SDS-PAGE.
A standard curve of the log (MW) versus R<sub>i</sub> was generated using the Precision Plus Protein standards from Figure 1. The strong linear relationship (\( r^2 > 0.99 \)) between the proteins’ MW and migration distances demonstrates exceptional reliability in predicting MW.
This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6040).