Imaging in-gel fluorescence and Stain-Free™ gels with the Azure c600

Detecting in-gel fluorescence enables the analysis of protein gels immediately after electrophoresis, without staining or Western blotting. Separation of proteins by electrophoresis is a cornerstone of protein analysis. After separation, identification of proteins typically requires several additional steps, either incubating the gel in a staining solution to stain the proteins, or transferring the proteins to a membrane for Western blotting and/or staining. Stain-Free™ gels incorporate compounds in the gel matrix that react with proteins upon exposure to UV light, generating fluorescent products that, with an appropriate imaging system, can be detected within the gel without further staining.

In-gel fluorescence detection also presents a timesaving approach to electrophoretic mobility shift or "gel shift" assays. The assays, frequently used to detect protein-nucleic acid interactions (Rio 2014), can also be used also detect protein-protein interactions. The technique is based on the fact that two proteins bound together in a complex will migrate differently through a native gel than will either protein alone. Binding is observed by staining the gel and detecting a band in a sample of a protein mixture that is not seen in samples of the individual proteins. Typically, proteins are transferred from the gel to a membrane for Western blotting to confirm the identities of the proteins in each band observed on the stained gel. A much faster and easier approach to studying protein-protein interactions by gel shift assays is to fluorescently label one of the proteins (Park, 2004). Then, after electrophoresis, the fluorescence can be detected directly in the gel, eliminating the need to stain the gel or to conduct a Western blot to identify new bands containing the protein of interest.

In this technical note, we present the use of the c600 to image a fluorescent gel-shift assay. Fluorescently labeled ubiquitin was incubated with the E1 ubiquitin-activating and E2 ubiquitin-conjugating enzymes, and the binding reactions analyzed by gel electrophoresis. First, free and bound ubiquitin were detected by capturing the in-gel fluorescence of the fluorescein-labeled ubiquitin. Then, total protein was detected fluorescently, without staining the gel or even removing it from the imager.

Methods

A series of incubations were conducted combining fluorescein-labeled ubiquitin and the E1 and E2 proteins. The binding reactions were quenched after 0.5, 1, 2, 4, 8, 16, 30, and 60 minutes by the addition of non-reducing SDS buffer. The samples were then loaded on a Mini-PROTEAN® TGX Stain-Free™ precast gel (Bio-Rad) and electrophoresis was conducted under non-denaturing conditions. The samples in each lane contained approximately 200 ng of fluorescein-labeled ubiquitin.

After electrophoresis, fluorescein-labeled ubiquitin in the gel was detected by imaging in the Azure c600, using the epi blue light source and filter in the gel acquisition mode. A 6–second exposure was captured.

After the fluorescein image was captured, total protein was imaged per instructions for Stain-Free gels. Briefly, using the UV transilluminator of the Azure c600, the gel was exposed to UV light at 302 nm for 5 minutes to cross-link trihalo compounds within the gel to tryptophan residues in proteins. Fluorescent protein bands were then detected by imaging the gel using the UV 365 nm light source and filter in the gel acquisition mode. A 10-second exposure was captured.

Results

The binding reactions were loaded onto a gel and proteins separated by gel electrophoresis under non-denaturing conditions. In-gel fluorescent imaging of the gel reveals free fluorescein-labeled ubiquitin and a time-dependent increase in both E1-ubiquitin and E2-ubiqitin complexes (Figure 1).

After detecting the in-gel fluorescence of fluorescein, the gel was left in the Azure c600 instrument and exposed to UV light at 302 nm to crosslink the trihalo compounds in the Stain-Free gel to tryptophan residues in the proteins.

Once crosslinking was complete, the gel was imaged to detect total protein. The presence of free E1 and E2 proteins in the reaction mixtures can be detected, as well as the E1-ubiquitin and E2-ubiquitin complexes that were seen when the gel was imaged for fluorescein (Figure 2).

Conclusions

The Azure c600 serves as a "one-stop shop" for fluorescent gel-shift assays. Using Stain-Free gels, both fluorescently labeled protein and total protein can be imaged without removing the gel from the imaging system. The resulting images can be overlaid to reveal which bands contain the fluorescently labeled protein, alleviating the need to stain the gel or conduct time-consuming Western blots.

Acknowledgements

Azure would like to thank Chris Balak, Dr. Jesse Hunter, and Dr. Lisa Baumbach-Reardon of the Integrated Cancer Genomics Division of the Translational Genomics Research Institute (TGen) for permission to use these data.

References

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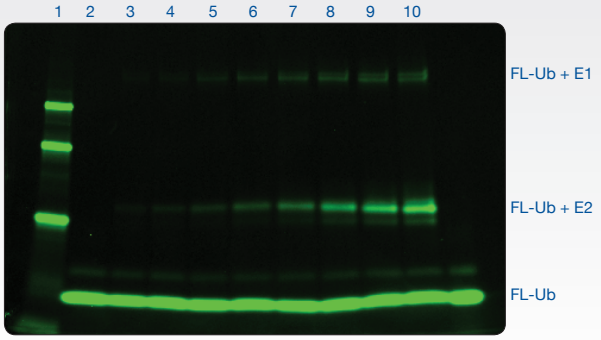

Figure 1. Fluorescent gel-shift assay using fluorescein-labeled ubiquitin (FL-Ub). FL-Ub, E1, and E2 were incubated for the indicated times and the reactions quenched with non-reducing SDS buffer. E1 and E2 bands with increasing fluorescence indicate covalent binding of fluorescent-labeled ubiquitin to E1 and E2. Fluorescein fluorescence was detected in the Azure c600 using excitation at 470 nm and emission filter at 497 nm. Lane 1: MW marker; lane 2: 0 min; lane 3: 0.5 min; lane 4: 1 min, lane 5: 2 min; lane 6: 4 min; lane 7: 8 min; lane 8: 16 min; lane 9: 30 min; lane 10: 60 min.

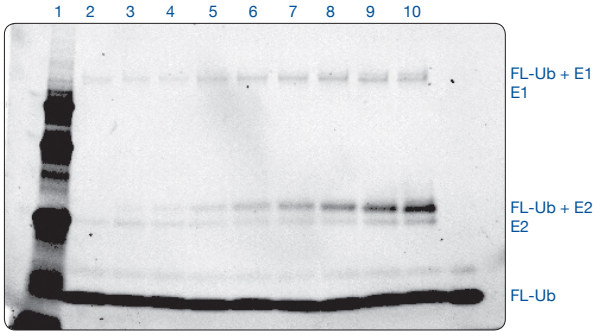

Figure 2. In-gel fluorescence imaging of total protein. The gel in Figure 1 was cross-linked for 5 minutes with the UV transilluminator in the Azure c600 imaging system, and then total protein detected in the UV365 channel. Lane 1: MW marker; lane 2: 0 min; lane 3: 0.5 min; lane 4: 1 min, lane 5: 2 min; lane 6: 4 min; lane 7: 8 min; lane 8: 16 min; lane 9: 30 min; lane 10: 60 min.

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