Simple Western: GFP Assay

Introduction

Green fluorescent protein (GFP) is an invaluable reporter for studying and engineering biological systems. This is particularly true in bioprocessing where protein expression platforms are engineered for the biosynthesis of protein products[1]. In upstream bioprocessing, when mammalian cells are transfected with recombinant DNA encoding the target product, GFP is routinely co-transfected as a marker to measure transfection efficiency. Furthermore, recombinant proteins produced in bioprocessing are often genetically fused with GFP as a biosensor to monitor expression, and to promote solubility and facilitate purification.

Sensitive detection of GFP in downstream bioprocessing is important because residual GFP contamination can cause undesirable immunogenic or cytotoxic effects[2], and immunodetection of GFP can reveal degradation or aggregation of GFP-tagged protein products. In this technical note, we describe how ProteinSimple's Simple Western™ enables bioprocess development with GFP. The workflow below describes two examples of how Wes™ works to streamline your bioprocess engineering workflows with GFP, including screening for residual GFP following co-transfection of host cells, and monitoring expression of GFP-tagged proteins in HEK 293 cells.

Materials

ProteinSimple

- Instrument: Wes
- 12–230 kDa Wes Separation Module (PN SM-W004)
- Anti-Goat Detection Module (PN DM-006)
- Bicine/CHAPS Lysis Buffer and Sample Diluent (PN 040-764)
- DMSO Inhibitor Mix (PN 040-510)
- Aqueous Inhibitor Mix (PN 040-482)

GFP reagents

- Goat anti-GFP antibody (R&D Systems, AF4240)
- Recombinant Jellyfish GFP (Novus Biologicals, NBC1-22949)

Basic GFP Assay Protocol

This protocol is a great start for any assay involving GFP detection.

1. Prepare 0.1X Sample Buffer by diluting the 10X Sample Buffer 1:100 in water.
2. Prepare a serial dilution series of the recombinant GFP in 0.1X Sample Buffer.

   In this example, we created a two-fold dilution series from 2 ng/mL to 0.125 ng/mL. Be sure to include a no-protein baseline control.
3. Dilute the anti-GFP antibody to 15 µg/mL in Milk-free Antibody Diluent.

   Note: *When using the anti-goat secondary antibody, use the Milk-free Antibody Diluent for preparing the antibody dilutions and as a blocking agent to avoid cross-reactivity. Milk-free Antibody Diluent is included in the Anti-Goat Detection Module and can be purchased separately (PN 043-524).*
4. Follow the default Wes sample preparation and assay conditions using the 12–230 kDa Wes Separation Module and the Anti-Goat Detection Module.
5. Generate a standard curve by plotting the GFP concentration of the serial dilution series by peak area.

Figure 1 shows the serial dilution series of recombinant GFP analyzed on Wes. This dilution series allows for the generation of a standard curve that can be used to determine the concentration of contaminating GFP in your unknown samples.

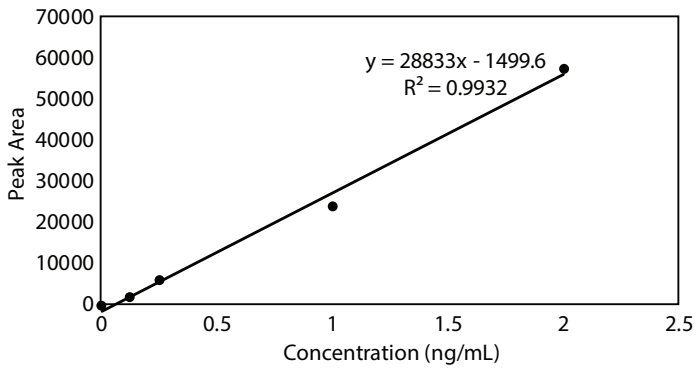

FIGURE 1. A serial dilution series of recombinant GFP analyzed on Wes. A serial dilution series from 2 ng/mL to 0.125 ng/mL was created in 0.1X Sample Buffer. The peak area is plotted for each concentration, and a line of fit was calculated. GFP was detected using a goat anti-GFP antibody.

Example 1: Measuring contaminating GFP

In upstream bioprocessing, GFP is often used to measure transfection efficiency. However, GFP is known to confer immunogenic and cytotoxic effects[2], so it may be undesirable if even a small residual amount of GFP lingers in downstream bioprocessing. Wes makes it easy to screen for contaminating GFP with sensitivity below 1 ng/mL. Here, we provide an example of how you can use a standard curve, like the one generated in **Figure 1**, to calculate the concentration of contaminating GFP in your unknown samples. The standard curve should be included in the same run as your unknown samples. **Figure 2** shows an example where there are two discrete bands representing two proteins, one of lower molecular weight (~30 kDa) and one of upper molecular weight (~150 kDa). The upper molecular weight band represents the expression of our target protein of interest fused to GFP, and the lower molecular weight band represents the residual free GFP in our samples following transfection. The concentration of the free GFP was calculated using the standard curve in **Figure 1**, and these values are listed below the gel view in **Figure 2**. This allowed us to detect very low levels of residual GFP, as little as 0.23 ng/mL.

Example 2: Screening GFP-tagged proteins

Tagging GFP to a target protein is a useful and common method to noninvasively monitor expression, subcellular localization, promote solubility, and facilitate purification. Wes quantitates GFP expression levels while also providing molecular weight information, allowing you to discern target proteins from degradation products or other impurities.

In this example, we analyzed a common bioprocess host, HEK 293, expressing a variety of recombinant proteins that are fused to GFP. Cells were lysed in Bicine/CHAPS Lysis Buffer with Aqueous Inhibitor/DMSO Inhibitor Mix for 30 minutes on ice. Samples were spun for 10 minutes at 15,000 x *g*, and just 0.3 µg of each sample was analyzed on Wes. GFP was detected using a goat anti-GFP antibody.

Figure 3 shows immunodetection of each protein using an anti-GFP antibody on Wes. Target protein products are outlined by green boxes. Bands appearing below the target protein products are likely the result of protein degradation while bands above the target protein product may be oligomers or higher order aggregates. Samples

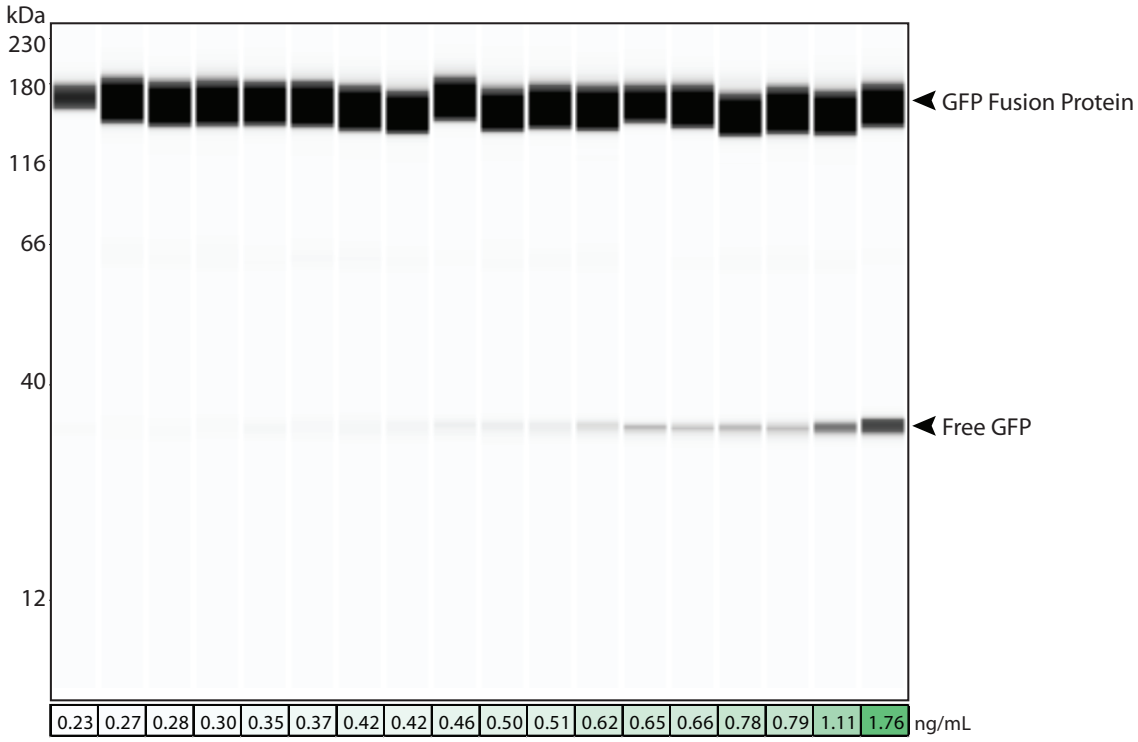

FIGURE 2. Free GFP contaminants in samples measured by Wes. The lanes were rearranged to sort by ascending free GFP. GFP was detected using goat anti-GFP antibody. The upper molecular weight band at ~150 kDa represents the protein of interest fused to GFP, and the lower molecular weight band at ~30 kDa is residual free GFP, indicated by the arrow. The concentration of the free GFP in each sample indicated by the arrow was calculated using the equation of the standard curve generated in **Figure 1**, and the values are listed below each corresponding lane. Shading of white to green corresponds to levels of low to high GFP, respectively.

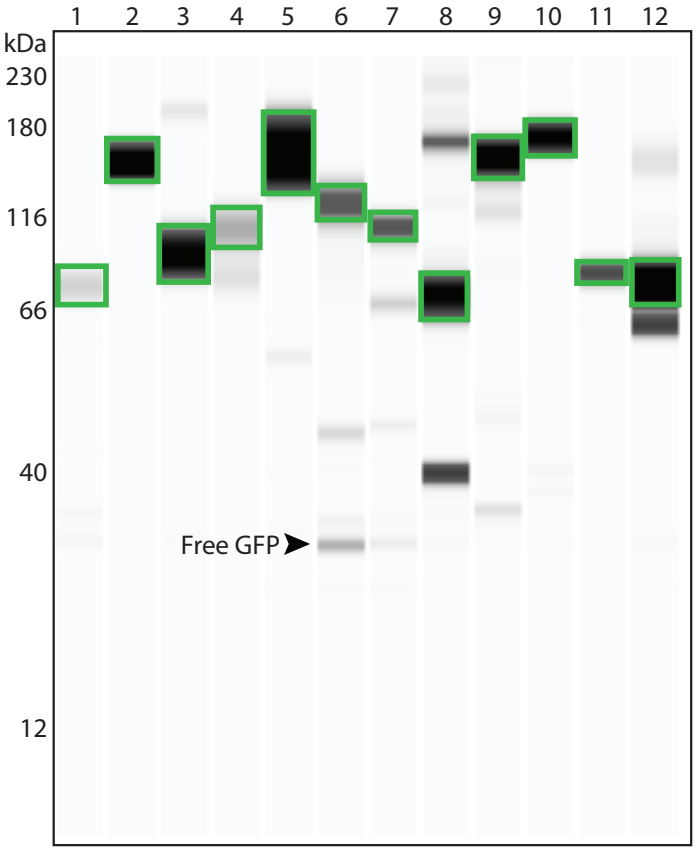

FIGURE 3. HEK 293 cells expressing a variety of recombinant proteins fused with GFP. Target protein products are outlined by green boxes. Free GFP is indicated by an arrow.

that contain such events are often undesirable and would be omitted from further investigation. Protein degradation or aggregation as measured by changes in molecular weight normally go undetected by flow cytometry or ELISA. By contrast, Wes allows you to rapidly identify these events without the need to run a time-consuming slab gel Western blot. Furthermore, the amount of free GFP in these samples may be calculated by using a standard curve like the one generated in **Figure 1**.

Conclusion

GFP is widely used in bioprocess engineering. Wes fits perfectly into your bioprocess engineering workflows by offering precise quantitation and molecular weight information of your GFP target in a short amount of time. Here we provide antibody and protocol recommendations and apply these recommendations to two independent bioprocess examples. Together, these examples demonstrate how Wes can provide concentration information not achievable by traditional Western blot and molecular weight information not achievable by FACS or ELISA, all without compromising sensitivity.

References

1. The green fluorescent protein is a versatile reporter for bioprocess monitoring, L Poppenborg, K Friehs and E Flaschel, *J Biotechnol*, 1997; 58(2):79-88.
2. Cellular GFP toxicity and immunogenicity: Potential confounders in in vivo cell tracking experiments, A Ansari, A Ahmed, A Matsangos, F Lay, L Born, G Marti, J Harmon and Z Sun, *Stem Cell Rev*, 2016; 12(5):553-59.

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