# Complete Workflow Automation for High-Throughput Western Blotting in Drug Discovery

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## ABSTRACT

ProteinSimple's automated western blotting system Jess can be used in a variety of applications, e.g. in drug discovery for applications like targeted protein degradation (TPD). With Analytik Jena's CyBio FeliX Liquid Handler for protein quantification setup, normalization, and Jess plate preparation, workflow efficiency can be significantly improved. PROTAC THAL-SNS-032 and its target molecule CDK-9 were used to demonstrate the automation capabilities. The quality of the automated process performed with the CyBio FeliX is as good as that of the manual process, while saving the scientist up to 60 minutes of time per Jess run.

## INTRODUCTION

Jess is a capillary based automated Western blot instrument capable of screening 24 samples in approximately 5 hours. Automation of the protein separation and immunodetection process eliminates error prone steps, and the associated software quickly processes the quantifiable data. At Charnwood Discovery, it is a staple technique in a repertoire of drug discovery methodologies. The well studied PROTAC THAL-SNS-032 was used to demonstrate the capabilities to further automate and simplify the Jess preparation procedure using the Analytik Jena CyBio FeliX liquid handling system.

## 1) ANTIBODY OPTIMIZATION

The anti-CDK9 antibody and loading control antibody ($\beta$-Actin) were optimized to ensure saturation and signal linearity, making data quantifiable. Using a stock cell lysate of predetermined protein concentration, the CyBio FeliX performs a serial dilution of the lysate, then prepares the Jess plate with varying antibody dilutions (Figure 1). Jess data analysis confirmed linear protein concentration-dependent detection of CDK9 and β-Actin (Figure 1B) as well as consistent signals indicating saturation across antibody dilutions (Figure 1C).

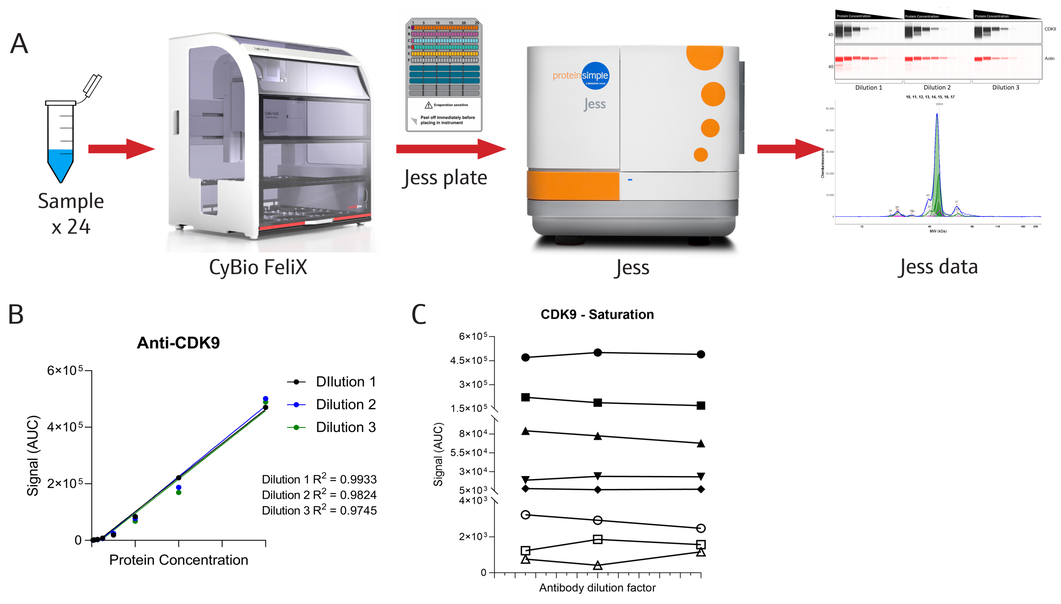


**Figure 1 |** (A) Schematic of antibody optimization. Samples with known target protein concentrations were used to determine and confirm linear signal response (B) as well as sufficient antibody saturation within the assay (C).

## 2) SAMPLE GENERATION & PROTEIN ASSAY

Cells were seeded and treated with serially diluted SNS-THAL-032 before being collected, washed with PBS, and then lysed with RIPA buffer.

Protein quantification ensures a concentration is used within the linear range of the antibody and helps to avoid camera saturation or ECL substrate depletion. The CyBio FeliX prepares the BCA assay using a BSA standard curve for extrapolation of unknown sample concentrations using absorbance at 562 nm (Figure 2).

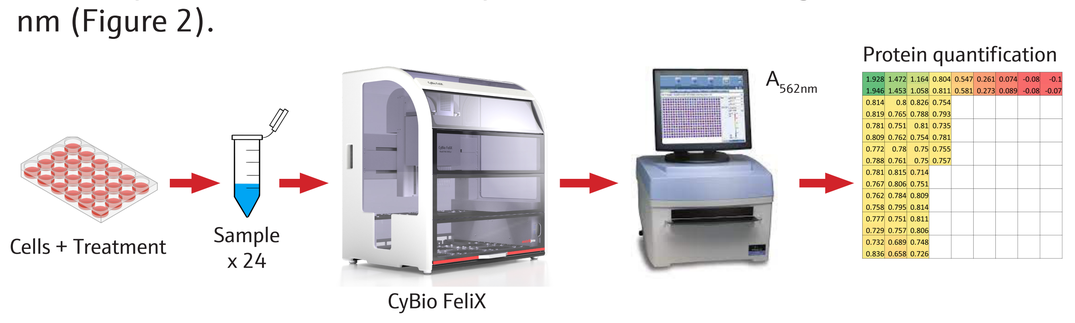


**Figure 2 |** Schematic of sampling and protein quantification. Total protein from cell lysate was quantified via BCA assay. The assay was prepared by the CyBio FeliX, the obtained protein concentration information was used to normalize samples during PROTAC assay setup.

## 3) PROTAC ASSAY: SNS-THAL-032 DOSE RESPONSE

Optimization performed in section 1 allows the protein of interest to be examined in the test samples. Files generated from the BCA assay in section 2 were used by the CyBio FeliX to prepare the Jess plates. Three biological replicates were measured to determine reproducibility of Jess plate preparation (Figure 3A). Dose-dependent CDK9 degradation by SNS-THAL-032 ($EC_{50}$=16.1±7.6 nM) was consistent across multiple experiments (Figure 3B). The quality of the automated process executed by the CyBio FeliX is the same as that of the manual process (Figure 3C), while saving a scientist up to 60 minutes of bench time per Jess run.

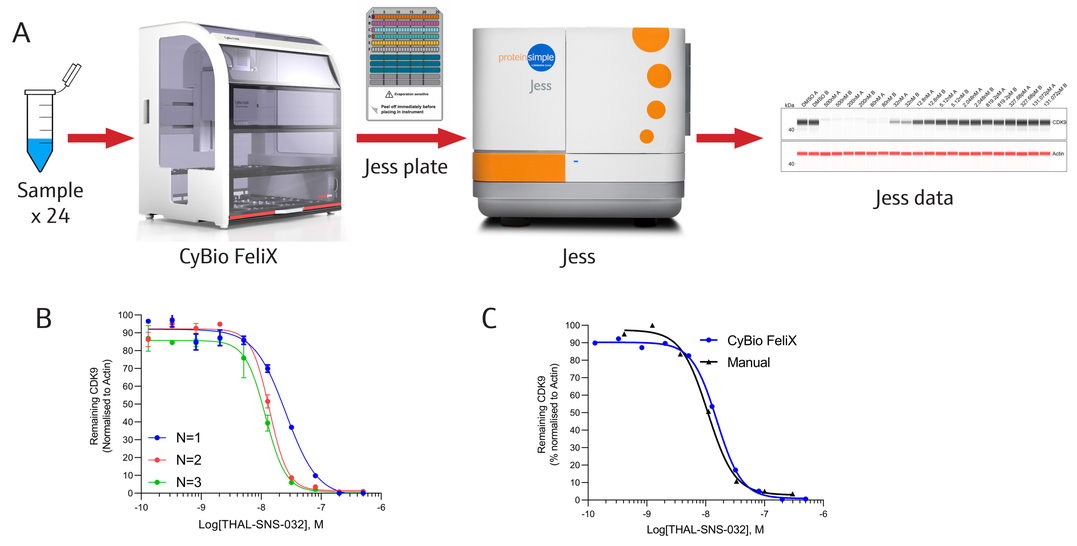


**Figure 3 |** (A) Schematic of the PROTAC assay. Samples with protein concentrations determined in section 2 were prepared for Jess analysis. CyBio FeliX uses protein quantification data to normalize sample protein concentrations. Triplicate analysis of SNS-THAL-032 mediated CDK9 degradation demonstrates reproducibility of the automated setup (B); its quality is identical to manual processing (C).

## CONCLUSION

Charnwood Discovery's Simple Western expertise using Jess combined with Analytik Jena's liquid handling automation expertise led to the development of a fully automated Western blotting method. The CyBio FeliX sample processing reduces bench time by around 60 minutes per Jess run, or up to 45 hours of bench time per week. Using the PROTAC THAL-SNS-032 to degrade CDK9, it was demonstrated that the CyBio FeliX can be used to optimize antibody & sample protein concentrations and prepare ProteinSimple Jess plate for screening purposes.

For more information check out our detailed solutions website.



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