



An Automated Method for the Capture and Detection of Epitope-tagged Proteins

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Abstract

Traditionally, enzyme linked immunosorbent assays (ELISA) of epitope-tagged proteins use micro-well plates passively coated with monoclonal antibodies. This can add several hours to an ELISA protocol. The ANTI-FLAG® High Sensitivity plates are coated with the mouse monoclonal M2 antibody and pre-blocked to provide timesavings for high-throughput users. The M2 monoclonal antibody is covalently bound to the plate so that the Fab region of the antibody is available for the epitope tag to provide specificity. A unique automated method has been developed for high-throughput ELISA applications using the Sciclone ALH 3000 Liquid Handling Workstation. This method provides walk-away automation for bacterial cell lysis, protein binding, and ELISA in the ANTI-FLAG micro-well plate. The data is shown to demonstrate the full functionality of this method to detect a protein of interest. In addition to ELISA, this method is well suited for such applications as screening for expression, as well as protein-protein interaction studies.

Materials

Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich (Saint Louis, MO). Cultures of *E. coli* were grown in Terrific Broth liquid media (T 5574). Purified Amino-terminal FLAG-Bacterial Alkaline Phosphatase or FLAG-BAP™ protein (P 7582) was used to create the standard curve. Rabbit Anti-BAP monoclonal antibody and anti-rabbit peroxidase conjugate (A 4312) were used to detect the FLAG-BAP protein. 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for ELISA (T 0440) substrate was used to quantify the protein of interest in each well.

Methods

Bacterial Growth and Protein Expression:

Bacteria were grown per standard conditions. IPTG was used to express the FLAG-BAP protein. Bacterial cells were transferred to a 96-well deep well plate for processing on the automated method for the Sciclone ALH 3000 Liquid Handling Workstation.

ELISA Method:

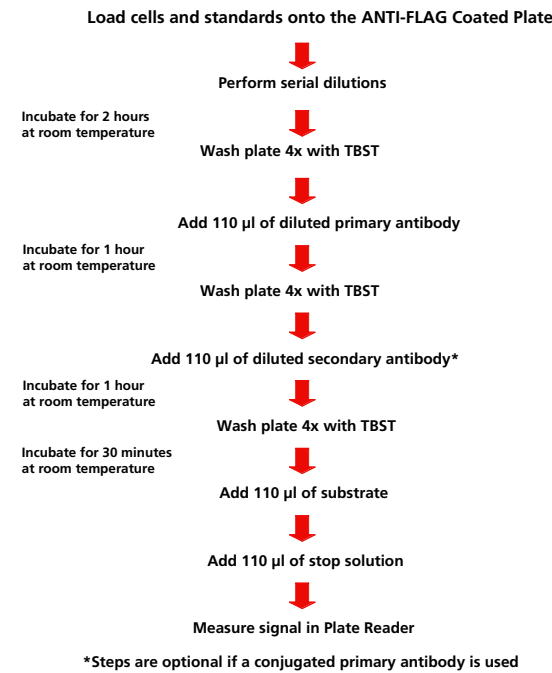
Purified FLAG-BAP protein (1000 ng) was serially diluted 1:3 in the top rows of the ANTI-FLAG® High Sensivity, M2 coated 96-well plate. In the bottom rows the cell lysate was serially diluted 1:10. The plate was then incubated with shaking for 2 hours. The plate was washed with TBST to remove non-specific proteins and cellular debris. The plate was then incubated with a 1:40,000 dilution of Rabbit Anti-BAP antibody followed by an incubation with a 1:80,000 dilution of Anti-Rabbit Peroxidase conjugate. 110 µl of TMB was added, and the plate was incubated with shaking for 30 minutes. The reaction was stopped using 110 µl of ELISA TMB stop buffer and the plate was read at 450 nm on a Molecular Devices SpectraMax® Plus³⁸⁴ plate reader.

	1	2	3	4	5	6	7	8	9	10	11	12
A	900 ng FLAG-BAP	300 ng FLAG-BAP	100 ng FLAG-BAP	33.3 ng FLAG-BAP	11.1 ng FLAG-BAP	3.7 ng FLAG-BAP	1.2 ng FLAG-BAP	0.4 ng FLAG-BAP	0.1 ng FLAG-BAP	0.03 ng FLAG-BAP	0.01 ng FLAG-BAP	0 ng FLAG-BAP
B	900 ng FLAG-BAP	300 ng FLAG-BAP	100 ng FLAG-BAP	33.3 ng FLAG-BAP	11.1 ng FLAG-BAP	3.7 ng FLAG-BAP	1.2 ng FLAG-BAP	0.4 ng FLAG-BAP	0.1 ng FLAG-BAP	0.03 ng FLAG-BAP	0.01 ng FLAG-BAP	0 ng FLAG-BAP
C	900 ng FLAG-BAP	300 ng FLAG-BAP	100 ng FLAG-BAP	33.3 ng FLAG-BAP	11.1 ng FLAG-BAP	3.7 ng FLAG-BAP	1.2 ng FLAG-BAP	0.4 ng FLAG-BAP	0.1 ng FLAG-BAP	0.03 ng FLAG-BAP	0.01 ng FLAG-BAP	0 ng FLAG-BAP
D	undiluted Cells in Media	1:10 dilution	1:100 dilution	1:1000 dilution	1:10000 dilution	1:100000 dilution	1:10 ⁶ dilution	1:10 ⁷ dilution	1:10 ⁸ dilution	1:10 ⁹ dilution	1:10 ¹⁰ dilution	1:10 ¹¹ dilution
E	undiluted Cells in Media	1:10 dilution	1:100 dilution	1:1000 dilution	1:10000 dilution	1:100000 dilution	1:10 ⁶ dilution	1:10 ⁷ dilution	1:10 ⁸ dilution	1:10 ⁹ dilution	1:10 ¹⁰ dilution	1:10 ¹¹ dilution
F	undiluted Cells in Media	1:10 dilution	1:100 dilution	1:1000 dilution	1:10000 dilution	1:100000 dilution	1:10 ⁶ dilution	1:10 ⁷ dilution	1:10 ⁸ dilution	1:10 ⁹ dilution	1:10 ¹⁰ dilution	1:10 ¹¹ dilution
G	undiluted Cells in Media	1:10 dilution	1:100 dilution	1:1000 dilution	1:10000 dilution	1:100000 dilution	1:10 ⁶ dilution	1:10 ⁷ dilution	1:10 ⁸ dilution	1:10 ⁹ dilution	1:10 ¹⁰ dilution	1:10 ¹¹ dilution
H	undiluted Cells in Media	1:10 dilution	1:100 dilution	1:1000 dilution	1:10000 dilution	1:100000 dilution	1:10 ⁶ dilution	1:10 ⁷ dilution	1:10 ⁸ dilution	1:10 ⁹ dilution	1:10 ¹⁰ dilution	1:10 ¹¹ dilution

ANTI-FLAG 96-well Plate Map

ANTI-FLAG 96-well Plate Map. The plate map above shows the dilutions of the purified FLAG-BAP protein standard (row 1-3) and Unknown lysates. The 1:1000 was the optimal dilution of the lysate within the linear range of the generated standard curves.

Automated ANTI-FLAG Plate ELISA Protocol



Automated Workstation



Figure 1. Sciclone ALH 3000 Liquid Handling Workstation. The automated ELISA method utilizing the ANTI-FLAG HS M2. Coated plates was developed and validated for the Sciclone ALH 3000. The system used in these experiments was configured with 96-Channel. High Volume Head, Z8™ Pipettor, Gripper, and a Bulk Dispense Module with syringe pump.

Validation of the Automated Method using the ANTI-FLAG M2 96-well plate

Standard Curve (Manual Method)

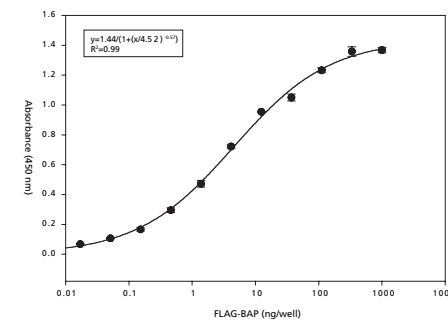


Figure 2: Standard curve analysis of FLAG-BAP protein. The standard curve was generated to determine the amount of FLAG-BAP protein in the induced *E. coli* lysate. Known concentrations of FLAG-BAP protein were serially diluted as described in the methods section. A best fit curve was generated using Sigmaplot™ and was subsequently used to quantitate the unknown lysate.

Standard Curve (Automated Method)

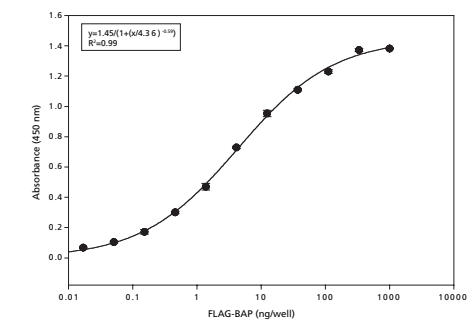


Figure 3: Standard curve analysis of FLAG-BAP protein. The standard curve was generated to determine the amount of FLAG-BAP protein in the induced *E. coli* lysate. Known concentrations of FLAG-BAP protein were serially diluted as described in the methods section. A best fit curve was generated using Sigmaplot™ and was subsequently used to quantitate the unknown lysate. The ELISA was performed using the Sciclone ALH 3000.

Protein Determination of FLAG-BAP Protein

Panel A: Protein Determination: Manual Method

	Absorbance	Mean	Total Protein*	Concentration
Replicate 1	1.08	1.05	25.8 µg	0.26 µg/µl
Replicate 2	1.07			
Replicate 3	1.08			
Replicate 4	1.01			
Replicate 5	1.03			

* $y = (1.44 / (1 + (x / 4.52)^{-1.12})) * 1000$

Panel B: Protein Determination: Automated Method

	Absorbance	Mean	Total Protein*	Concentration
Replicate 1	1.09	1.05	24.8 µg	0.25 µg/µl
Replicate 2	1.00			
Replicate 3	1.00			
Replicate 4	1.00			
Replicate 5	0.98			

* $y = (1.43 / (1 + (x / 4.53)^{-1.12})) * 1000$

Figure 4: Protein Quantification of FLAG-BAP protein dilution. The concentration of FLAG-BAP protein in cell lysates was determined using a manual method and the automated method developed on the Sciclone. The Calculated values for both methods were within 5% of each other.

Cross Contamination Analysis

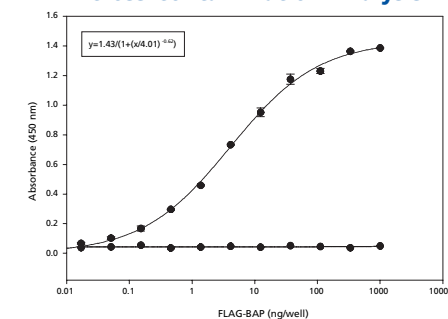


Figure 5: Cross contamination analysis. The automated method for running the ELISA was tested for the potential of cross-contamination. This was evaluated by leaving blank rows between rows of FLAG-BAP protein standards. The figure above demonstrates that no cross contamination was detected in the negative controls.

Conclusions

- Data demonstrates the effectiveness of ANTI-FLAG M2 96-well plates to capture and detect a FLAG-tagged protein of interest in an ELISA format
- Data demonstrates the high sensitivity and binding capacity of the ANTI-FLAG HS M2 Coated Plates
 - As little as 1 ng/well of protein can be detected
 - Plates have a binding capacity of 300 ng/well
- The walk-away automated protocol for the ANTI-FLAG M2 plate enables high throughput protein expression detection via an ELISA
 - The entire process is automated from addition of cells lysates through introduction of a stop solution
 - Plates are pre-blocked to offer a significant time-savings to high-throughput users