Anti-c-Myc Coated HS Multiwell Plates
Format: 96-well, clear

Product Number **P 2241**

ProductInformation

TECHNICAL BULLETIN

Product Description
Expression vectors that encode the DNA sequence for specific non-native proteins provide convenient means for producing and purifying proteins of interest in a foreign organism. These expression vectors also allow specific peptide or protein sequences to be fused with the cloned protein. These protein fusion partners can help stabilize the expressed protein and/or increase its solubility. Fusion partners can also serve as affinity handles or tags, such as FLAG®, c-Myc or poly-histidine, that facilitate the capture, purification and detection of the recombinant protein.[1-5]

The Anti-c-Myc Coated HS Multiwell Plate is a versatile platform designed for the capture and detection of recombinant c-Myc fusion proteins isolated from c-Myc protein expression systems. Anti-c-Myc, a mouse monoclonal IgG_1 antibody (clone 9E10), is covalently linked to the surface of a multiwell plate via the Fc portion of the antibody. This linkage provides a favorable orientation of the antibody on the surface of the plate affording higher binding capacity compared to that of a passively absorbed antibody surface.

Following transformation, growth, expression, and lysis of a host organism, ELISA, SDS-PAGE electrophoresis, Western Blot, MALDI MS, or affinity binding analysis may be used to assess the recombinant protein content. In the event that many cell samples containing c-Myc-linked fusion proteins are to be analyzed, the anti-c-Myc high sensitivity capture plate offers greater speed and convenience over other methods. These plates are also powerful tools in High Throughput Screening (HTS) applications. The plates may be used for general screening of recombinant protein production, protein/protein interaction studies, protein/organic molecule interaction studies, signal transduction studies, and/or estimation of fusion protein content.

Reaction Volume
Anti-c-Myc mouse monoclonal antibody, IgG_1, is coated at a reaction volume of 200 µl/well. To prevent non-specific binding, the wells are blocked with 300 µl of BSA per well.

Specificity
The plates are specific for the c-Myc epitope (the decapeptide Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) regardless of its placement in the fusion protein: N-terminal, Met-N-terminal, C-terminal or internal. Binding of the epitope is not Ca^{2+} dependent.

Sensitivity
Detection of ≤1 ng of a control fusion protein was observed in an ELISA format with p-nitrophenyl phosphate as a substrate.

Precautions and Disclaimer
For research use only. Not for use in diagnostic procedures. **Regeneration and reuse of the plate is not recommended.**

Storage/Stability
For optimal performance, the unopened product should be stored in a dry place at 2-8 °C. It may be stored at room temperature for up to 3 months, but should not be exposed to temperatures above 50 °C.
Once opened, the product should be used promptly.

Refer to the Certificate of Analysis for expiration date. The certificate can be obtained from the Sigma-Aldrich website (www.sigma-aldrich.com).

Procedures
Basic Binding and Elution of a c-Myc Fusion Protein; Immunoprecipitation with SDS Page analysis:

Materials Required
- P2241, Anti-c-Myc Coated HS 96-well Plate
- Cell lysate containing c-Myc fusion protein
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Equipment Required
- Multi-channel (8- or 12-channel) pipet
- Spectrophotometer capable of reading 96-well multiwell plates

Procedure

1. Load up to 200 µl/well of cell lysate containing the c-Myc fusion protein.

2. Cover with a 96-well plate cover or sealing tape (Product No. T 2162).

3. Incubate the cell lysate in the plate for a minimum of one hour at 37 °C or 2 hours at room temperature (18-30 °C), or overnight at 2-8 °C.

4. Remove the plate cover and wash the plate three times (300 µl per well) with an appropriate wash buffer (e.g. 100 mM Tris buffered saline, pH 8.2, containing 2 mM $MgCl_2$, 0.5% TWEEN®20, for Alkaline Phosphatase conjugates).

5. Elute the protein by loading 30 µl of a 2X sample buffer containing 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue and 125 mM Tris-HCL, pH 6.8 (Product No. S 3401).

   Note: Any one of several common elution buffers may be used to elute the c-Myc fusion proteins from the surface of the plate. The buffer described above is commonly used for preparation of c-Myc fusion proteins for SDS-PAGE analysis.

6. Seal the plate with sealing tape (Product No. T 2162).

7. Vortex for a minimum of 10 minutes, making certain that the buffer completely washes the sides of the wells. Caution: ensure that the buffer does not splash from the plate.

8. Remove samples from wells and pipette into 0.5 ml Eppendorf tubes. Heat samples for 2 min at 90 °C or load samples directly onto an SDS-PAGE gel and run per the manufacturer's recommendations.

9. Gels may be stained (with silver stain, Product Code AG-5 or EZ-Blue Stain, Product No. G 1041), or Western Blot analysis may be performed.

Basic Cell Lysate Screening

Materials Required

- P2241, Anti-c-Myc Coated HS 96-well Plate
- Cell lysate containing c-Myc fusion protein
- Antibody against the fusion protein of interest and a labeled secondary antibody, if necessary.
- Substrate for the detection of enzyme.

Equipment Required

- Multi-channel (8- or 12-channel) pipet
- Spectrophotometer capable of reading 96-well multiwell plates

Procedure

1. Prepare serial dilutions of the crude cell lysate containing the c-Myc fusion protein with a dilution buffer of choice (e.g. phosphate or Tris buffered saline containing 0.5% TWEEN® 20).

   Note: Initially, it is recommended that log dilutions of the cell lysate be done to determine the performance characteristics of the expression system.

2. Apply up to 200 µl per well of each diluted lysate, or, as a negative control, apply up to 200 µl per well of non-expression cell lysate.

3. Cover with a 96-well plate cover (Product No. Z37189-0) or sealing tape (Product No. T 2162)

4. Incubate the cell lysate in the plate for a minimum of one hour at 37 °C or 2 hours at room temperature (18-30 °C), or overnight at 2-8 °C.

5. Remove the plate cover and wash the plate three times (300 µl per well) with an appropriate wash solution (e.g. phosphate or Tris buffered saline containing 0.5% TWEEN® 20).

   Note: Plate washing may be done with an automated plate washer or by hand with a multi-channel pipet. If washing by hand, remove residual liquid by inverting the plate and gently tapping it on lint-free paper towels.

6. Prepare a dilution of the primary antibody or antibody-enzyme conjugate using an appropriate diluent (e.g. 100 mM Tris buffered saline, pH 8.2, containing 1% BSA, 2 mM $MgCl_2$, and 0.5% TWEEN® 20, for Alkaline Phosphatase conjugates).

   Note: For detection using an antibody against the fusion protein, the primary and secondary antibody dilutions must be determined empirically.

7. Apply a volume of the diluted antibody that is in small excess to the original cell lysate volume (e.g. if 100 µl/ well of cell lysate was initially loaded, load 125 µl of the primary or secondary antibody per well). Cover the plate with a 96-well plate cover or sealing tape.

8. Incubate the plate for a minimum of one hour at room temperature (18-30 °C).

   Note: Proceed to step 13 if an antibody-enzyme conjugate was used in steps 6-8. If an unlabeled primary antibody was used in steps 6-8, proceed to step 9

9. Remove the plate cover and wash the plate three times (300 µl per well) with an appropriate wash solution.

10. Prepare a dilution of the secondary antibody-enzyme conjugate using an appropriate diluent (e.g. 100 mM Tris buffered saline, pH 8.2, containing 1% BSA, 2 mM $MgCl_2$, and 0.5% TWEEN 20, for Alkaline Phosphatase conjugates).

11. Apply a volume of the diluted secondary antibody that is equal to the volume of primary antibody used in Step 8. Cover the plate with a 96-well plate cover or sealing tape.

12. Incubate the plate for a minimum of one hour at room temperature (18-30 °C).

13. Remove the plate cover and wash the plate three times (300 µl per well) with an appropriate wash solution (e.g. 100 mM Tris buffered saline, pH 8.2, containing 2 mM $MgCl_2$, 0.5% TWEEN® 20, for Alkaline Phosphatase conjugates)

Detection of Bound Fusion Proteins

1. Prepare any necessary substrate and/or stop solutions per product instructions (e.g. pNPP for alkaline phosphatase, Product No. N 7653).
2. Apply up to 200 µl of the appropriate substrate per well.
3. Using a multiwell plate reader, record signal per substrate manufacturer's recommendations (e.g. 405 nm for pNPP).

Results

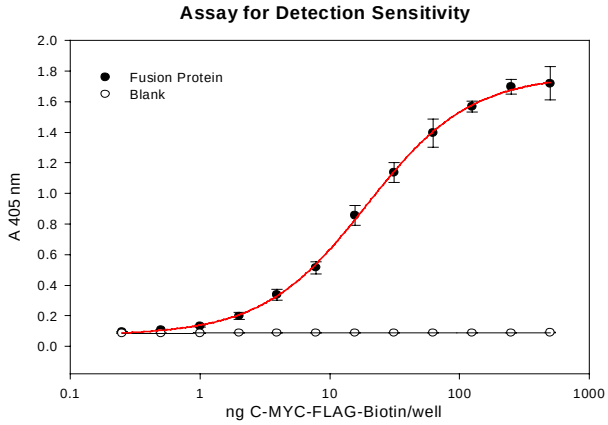

Plate Features: (format)

Property	96-Well Plate
Plate composition	Virgin polystyrene
Lid	Yes
Well configuration	Flat bottom/round
Well width	6.4 mm
Well depth	11 mm
Maximum recommended working volume, per well	200 µL
Coating coefficient of variability, well-to-well	≤ 10%

References

1. Current Protocols in Molecular Biology, Ausubel, F.M., et al., John Wiley and Sons Inc., N.Y., 1998, pp. 10.15.1-10.16.29
2. Antibodies, A Laboratory Manual, Harlow E. and Lane D., Cold Spring Harbor Laboratory Press, NY, 1988, pp. 514-517, 541-542, 547-549
3. Fan H. et al., Biochem. Cell Biol., **76(1)**, 125 (1998)
4. Robertson, D., et al., J. Histochem. Cytochem., **43,** 471-480 (1995).
5. Kleymann, G., et al., J. Histochem. Cytochem., **43,** 607-614 (1995).

Results Troubleshooting Guide

Screening

Problem	Possible Cause	Solution
High Background	Crude cell lysate may contain enzymes capable of turning over substrate	Check the negative control, non-expressing cell lysate
	Antibody titer too high	Increase dilution of primary and/or secondary antibodies
	Substrate incubation time too long or substrate is degraded	Decrease time exposed to substrate or utilize a fresh substrate
No signal	No fusion protein expressed	Re-evaluate the expression system and prepare a fresh culture for analysis
	Antibody/Antibody-enzyme conjugate inactive	Utilize a proven antibody/antibody-enzyme conjugate at appropriate titers
	Degraded substrate	Utilize a fresh stock of substrate
Low Signal	Low level of fusion protein	Re-evaluate the expression system and prepare a fresh culture for analysis
	Crude cell lysate too dilute	Reduce the lysate dilution factor
	Antibody titer too low	Reduce the dilution of primary and/or secondary antibodies
	Substrate incubation time insufficient	Increase exposure to substrate as long as background levels remain low
High Signal	Crude cell lysate too concentrated	Further dilute crude cell lysate
	Antibody titer too high	Increase the dilution of primary and/or secondary antibodies
	Substrate incubation time too long	Decrease exposure time to substrate

Immunoprecipitation

Problem	Possible Cause	Solution
Low Yield of Eluted Protein	Low Binding Efficiency	Increase the amount of lysate/protein in the binding step and/or extend the binding duration to overnight.
	Low elution efficiency	Allow longer incubation with the elution buffer. Check to see that the agitation of the elution buffer is sufficient to completely wash the well walls

Reagent Compatibility Table

Reagent	Effect	Comments
Chaotropic agents (e.g. urea, guanidine HCl)	Denatures the immobilized anti-c-Myc antibody	**Do not** use any reagent that contains these types of components since it will denature the anti-c-Myc antibody and destroy its ability to bind the c-Myc tagged proteins. Low concentrations of urea (1 M or less) can be used.
Reducing agents (e.g. DTT, DTE, 2-mercaptoethanol)	Reduces the disulfide bridges in the anti-c-Myc antibody chains	**Do not** use any reagent that contains these types of components since it will reduce the disulfide linkages in the anti-c-Myc antibody and destroy its ability to bind the c-Myc tagged proteins.
TWEEN® 20, 5% or less Triton® X-100 5% or less IGEPAL® CA-630, 0.1% or less CHAPS, 0.1% or less Digitonin, 0.2% or less	Reduces non-specific protein binding	Do not exceed recommended concentration.
Sodium chloride, 1.0 M or less	Reduces non-specific protein binding by reducing ionic interactions	Do not exceed recommended concentration.
Sodium dodecyl sulfate (SDS)	Denatures the immobilized anti-c-Myc antibody	**Do not** use any reagent that contains this detergent in the loading and washing buffers since it will denature the anti-c-Myc antibody and destroy its ability to bind the c-Myc tagged proteins.
Deoxycholate	Interferes with anti-c-Myc binding to c-Myc proteins	**Do not** use any reagent that contains this detergent since it will inhibit the anti-c-Myc antibody from binding to c-Myc fusion proteins.

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