eCLIP Validated Antibodies

RBP-eCLIP Validated Antibodies from Eclipsebio & Cell Signaling Technology

What is eCLIP?

Enhanced **C**ross-**L**inking & **I**mmuno**P**recipitation **(eCLIP)** was developed in Professor Gene Yeo's Lab[1] for identifying RNA binding sites bound by RNA-binding proteins (RBPs). eCLIP has improved ligation efficiency & uses a size-matched input control.

Why Validated Antibodies Matter

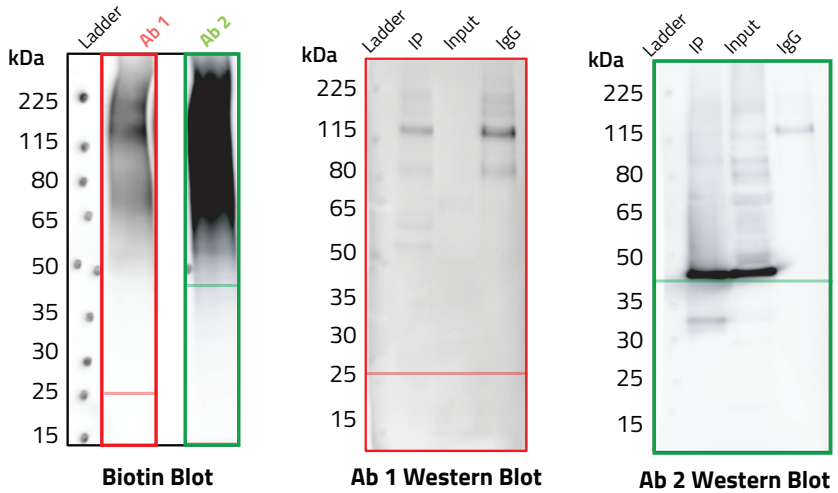
A successful eCLIP experiment is heavily dependent on the quality of the antibody. Commercial IP-validated antibodies tend to fail validation due to eCLIP's stringent washes. Eclipsebio & Cell Signaling Technology (CST) have partnered to expertly validate CST antibodies.

How Eclipsebio Validates CST Antibodies

Eclipsebio's Antibody IP Validation method assesses both IP efficiency (western blot) and RNA yield (biotin blot) after stringent eCLIP washes. Eclipsebio's validation standards are used both when selecting an antibody and during the actual eCLIP experiment.

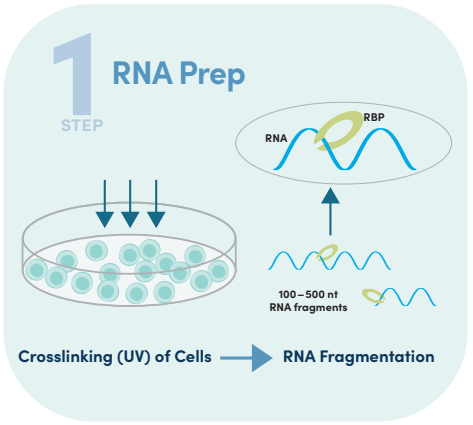
Failed Competitor "IP Grade" Antibody
Expected M.W. for Protein 1

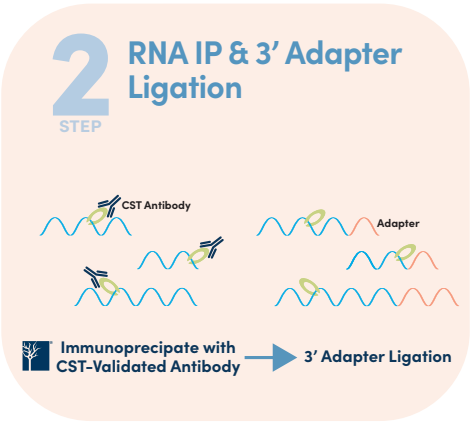
Passed CST Validated Antibody
Expected M.W. for Protein 2

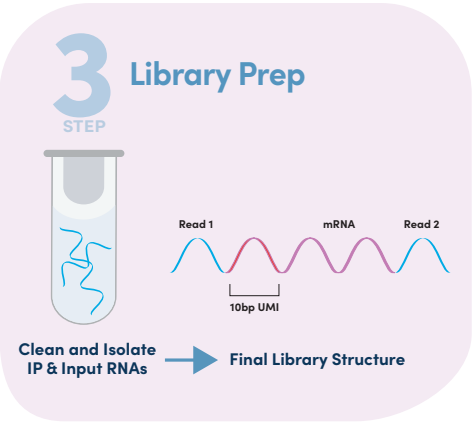


Western and biotin blot antibody validation data presented for 2 proteins of interest in HEK293 cells using Eclipsebio's Antibody IP Validation method. Ab 1 results in a failed validation test. Biotin blot data for Ab 1 indicates RNA smear begins at incorrect molecular weight (M.W.). Bands for Ab 1 are not present at the expected M.W. of the western blot data. CST-validated antibody, Ab 2, results in a passed validation test. Biotin blot data for Ab 2 contains robust RNA smears at the expected M.W. Bands for Ab 2 are present at the expected M.W. in the western blot data.

The RBP-eCLIP Workflow







RBP-RNA interactions are UV crosslinked. RNA is fragmented, and an RBP of interest is immunoprecipitated with a CST antibody. PCR amplification is then used to obtain sufficient material for highthroughput sequencing.

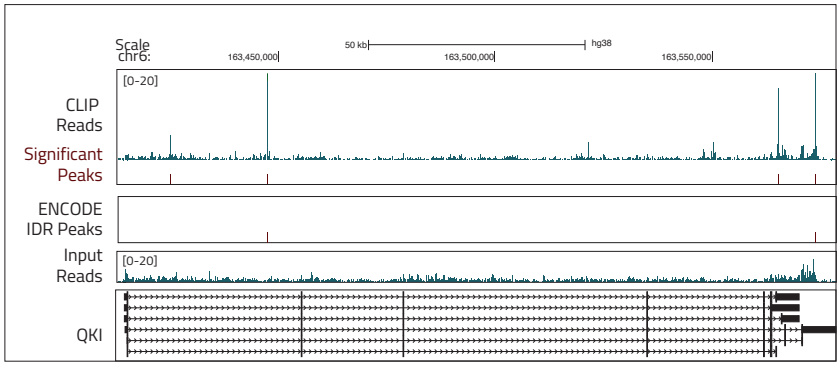
ECLIPSEBIO
THE RNA GENOMICS COMPANY

Cell Signaling TECHNOLOGY®

Methods to Confirm Antibody Specificity

eCLIP-identified targets can be verified in any of three ways:

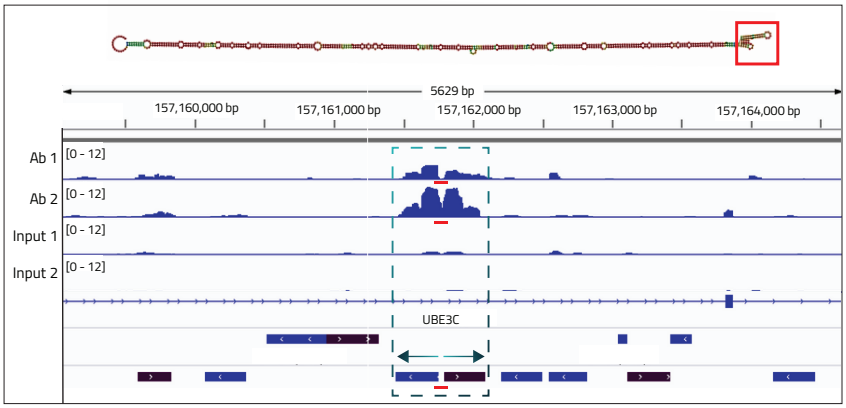
1. Compare results to published CLIP or RNA-IP-Seq data to confirm high-confidence RBP binding locations are identified as areas of significant read enrichment in the IP library compared to the control library.



eCLIP was performed with RNA from K562 cells and **CST QKI (E7O4A) Rabbit mAb #23065** using a protocol based on RBP-eCLIP from Eclipsebio. The figure shows an acceptable minimum number of defined enrichment peaks and a minimum signal:noise threshold compared to the input across the QKI transcript, with compared eCLIP data downloaded from ENCODE.

2. Execute an eCLIP experiment using antibodies against different RBPs within the same protein complex as the binding locations should be similar.

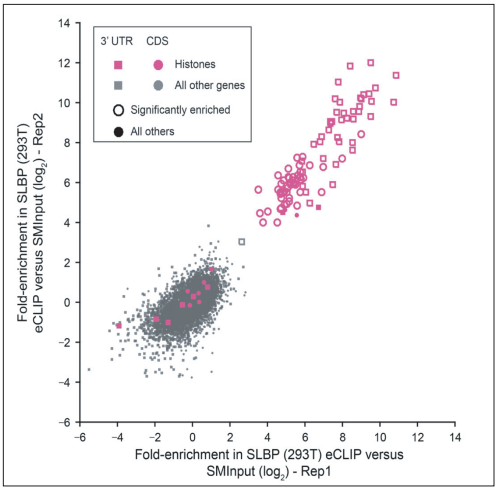
3. Perform an eCLIP experiment with antibodies against different epitopes within the same RBP to confirm similar binding locations.



Elevated p110 read densities on two adjacent, opposite stranded Alu elements with a prominent dip in coverage at the tip of a predicted hairpin. Antibodies 1 and 2 bind to different epitopes on p110 and their identified peaks strongly overlap and show similar characteristics, indicating a strong likelihood of a true positive binding event. The hairpin region is denoted by a red mark on the sequence tracks and corresponds with the hairpin region boxed on the folded RNA.

Antibody Reproducibility

Each antibody is tested in duplicate and must generate an acceptable Irreproducible Discovery Rate, as previously described for ChIP-Seq data analysis[2].



IP fold enrichment over control (SMInput) of histone RNAs using RBP-eCLIP against SLBP is reproducible across biological replicates. Each point indicates eCLIP fold-enrichment over paired SMInput for the CDS (circle) or 3'UTR (square) of genes profiled in independent biological replicate SLBP eCLIP experiments. Histone genes are indicated in pink, with open circles indicating significantly enriched regions (fold-enrichment ≥ 4-fold, p-value ≤ 10^{-5} in eCLIP vs SMInput). Both CDS (R^2 = 0.50) and 3' UTR (R^2 = 0.73) show significant correlation ($p < 10^{-300}$, all significance determined by standard conversion of r values to t-statistic), and show enrichment at most histones.

REFERENCES

1. Van Nostrand E.L., et al. (2016) Nat Methods. 13(6):508-14.

2. Li, Q., et al. (2011) Ann. Appl. Stat. 5(3),1752-1779.

For Additional Information:

Eclipsebio's RBP-eCLIP Services
Visit: **www.eclipsebio.com**

Cell Signaling Technology Antibodies
Visit: **www.cellsignal.com**