ENL (YEATS Domain) and Acyl-Modified Histone Binding Assay

A protein interaction assay to enable the identification of novel ENL inhibitors

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Overview: YEATS Domain Epigenetic Reader Proteins

Epigenetic reader proteins have gained widespread interest in the scientific community for their essential roles in cellular function and the potential for targeted drug development to treat diseases such as cancer. Recently, a novel family of reader proteins was identified that recognizes acetylated lysine (Kac) residues on histone tails through a highly conserved YEATS domain. In contrast to the widely studied Kac bromodomain reader, the YEATS domain also shows affinity for extended acyl modifications, particularly lysine crotonylation (Kcr) [1]. There are four YEATS domain containing proteins in humans: AF9, ENL, GAS41, and YEATS2. Due to the emerging role of these YEATS:acyl-histone interactions in cellular transcription and human disease [2], there is a compelling need to identify small molecule inhibitors of this novel class of epigenetic readers.

ENL YEATS Domain

Recent studies identify the ENL YEATS domain as a critical driver of oncogenic gene expression in acute myeloid leukaemia [3, 4]. ENL is required for leukaemic growth in vitro and in a mouse xenotransplant model. The role of ENL in RNA polymerase II-mediated transcription at oncogenes is specifically mediated by its YEATS acyl reader domain. These findings underscore the need to develop assays for the identification of novel inhibitors of ENL-YEATS interactions with modified histones.

A biochemical assay to quantify ENL YEATS:acyl-histone interactions

This assay, based on Perkin Elmer's AlphaScreen® technology, enables a quantitative assessment of the ENL YEATS domain interaction with acetylated or crotonylated histone peptides (Figure 1). The homogenous "mix-and-measure" nature, absence of wash steps, ability to miniaturize and automate the assay in multi-well plates, and robust nature of the approach make it ideal for high-throughput screening (HTS) applications.

As previously reported, ENL exhibits modest binding to histone H3 acetylated at lysine 9 or lysine 27 (H3K9ac or H3K27ac respectively; Figure 1B) [3, 4]. Moreover, ENL binding is enhanced by histone crotonylation over acetylation at the same sites (Figure 1B) [5], offering a variety of acyl-histone peptides that can be used to develop an interaction assay. Here, we show representative HTS assay development data for ENL binding to H3K27cr.

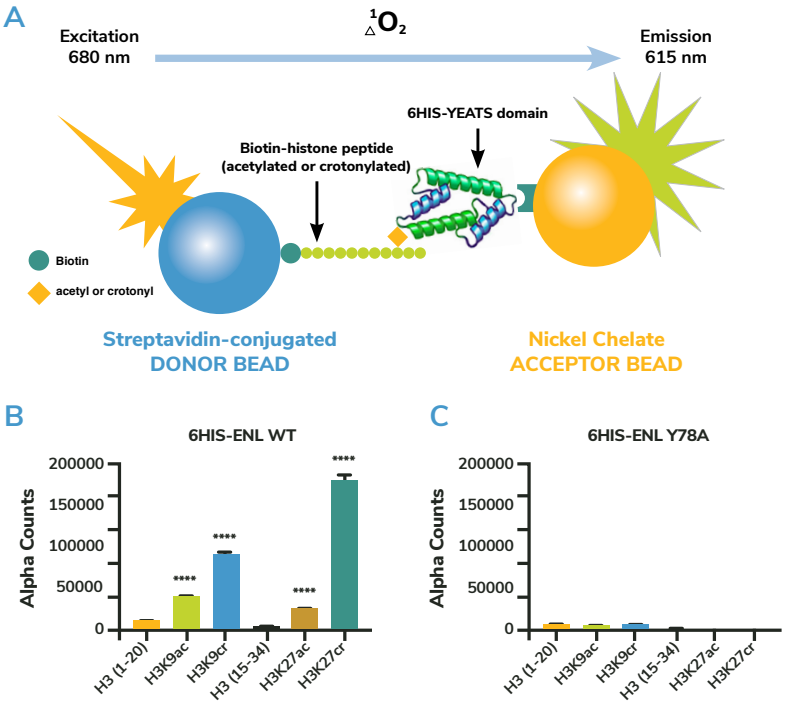

FIGURE 1: ASSAY PRINCIPLE. *An Amplified Luminescent Proximity Homogeneous Assay, or "Alpha" can be used to quantify histone binding to the YEATS reader domain.* (A) *A Streptavidin Donor Bead captures the biotinylated histone peptide. When the Donor Bead is excited at 680 nm, a short-lived singlet oxygen molecule is released. This causes emission from Nickel Chelate Alpha Acceptor Beads only when the histone peptide is bound to the 6HIS-tagged YEATS domain protein, thereby bringing the beads in close proximity.* (B) *This assay was optimized to quantify any interaction of the ENL YEATS domain with acetylated (ac, green bars) or crotonylated (cr, blue bars) histone peptides. ENL binding to the acyl-modified histone peptides is highly enriched over unmodified H3 peptide (black bars). White values on the bars indicate signal:baseline compared to unmodified H3 (****p<0.0001 compared to unmodified H3 using One-way ANOVA with Bonferroni post-test, n=9).* (C) *Point mutation of a conserved aromatic residue in the ENL YEATS domain (Y78A) previously identified to abolish binding to acyl lysines [3, 4] also abolishes the Alpha signal. WT = wild type ENL.*

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TECHNICAL NOTE #105

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Reagents and Materials Required for the Assay

NOTE: store protein aliquots @ -80°C, avoid freeze/thaw

- 6HIS-tagged Recombinant Human Proteins:
  - a. 6HIS-ENL YEATS Domain (Cat. No. 15-0069)
  - b. 6HIS-ENL YEATS Domain (Y78A) (Cat. No. 15-0070)
- Biotinylated Histone Peptides:
  - a. H3, aa 15-34, biotinylated (Cat. No. 12-0016)
  - b. H3K27cr, aa 15-34, biotinylated (Cat. No. 12-0100)
- Non-biotinylated Competitor Peptides:
  - a. H3, aa 15-34, (Cat. No. 12-8104)
  - b. H3K27cr, aa 15-34, (Cat. No. 12-8100)
- AlphaScreen Histidine Detection Kit (Perkin Elmer #6760619) containing:
  - a. AlphaScreen Nickel Chelate Acceptor Beads (5 mg/ml)
  - b. AlphaScreen Streptavidin Donor Beads (5 mg/ml)
- Assay Buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% BSA; add 0.1% NP-40 fresh)

  *DO NOT ADD A REDUCING AGENT
- AlphaPlate-384™, Light gray (Perkin Elmer #6005350)
- TopSeal™-A Plus Film (Perkin Elmer #6050185)

Standard Protocol

NOTE: Briefly centrifuge plate (200 rcf, 1 sec) after the addition of each reagent to maximize reproducibility.

NOTE: AlphaScreen Donor Beads are light sensitive. Work under subdued lighting.

1. Add 4 µl of 5X test inhibitors, competitor peptides, or vehicle control
2. Add 4 µl 5X 6HIS-ENL (80 nM final)
3. Incubate 15 minutes at RT
4. Add 4 µl 5X Biotin-H3 peptides (40 nM final)
5. Incubate 30 minutes at RT
6. Add 8 µl 2.5X Nickel Chelate Acceptor Beads + Streptavidin Donor Beads (1:100 dilution from stocks for 20 µg/mL final in 20 µl assay volume) under subdued lighting
7. Incubate 60 minutes at RT in the dark
8. Read using Alpha Plate Reader: Ex 680 nm, Em 615 nm

References

1 Sabari BR, Zhang D, Allis CD, Zhao Y. (2017) Metabolic regulation of gene expression through histone acylations. Nat Rev Mol Cell Biol 18(2):90-101.

2 Schulze JM, Wang AY, Kobor MS. (2009) YEATS domain proteins: a diverse family with many links to chromatin modification and transcription. Biochem Cell Biol 87(1):65-75.

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5 Li Y, Sabari BR, Panchenko T, Wen H, Zhao D, Guan H, Wan L, Huang H, Tang Z, Zhao Y, Roeder RG, Shi X, Allis CD, Li H. (2016) Molecular Coupling of Histone Crotonylation and Active Transcription by AF9 YEATS Domain. Mol Cell 62(2):181-93.

6 Zhang JH, Chung TD, Oldenburg KR. (1999) A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen 4(2):67-73.

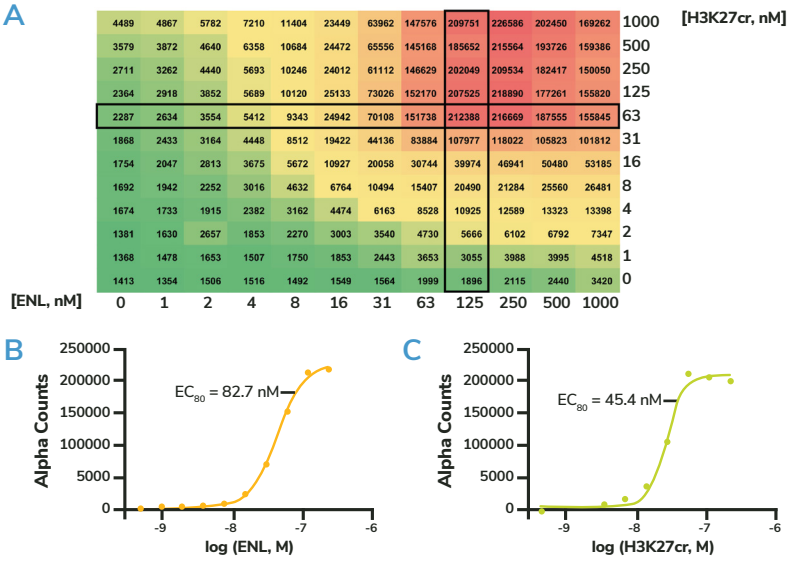

FIGURE 2: PROTEIN/PEPTIDE TITRATIONS. *(A) 6HIS-ENL (horizontal axis) was titrated against Biotin-H3K27cr peptide (vertical axis). Titrations of each binding partner from 1000 nM to 1 nM (1:2 dilutions) determined the optimal concentrations for assay development. Alpha Counts are shown and colored by relative intensity. The boxed regions outline the ENL/histone titrations graphed below. Nonlinear regression analysis identified the concentration at which 80% response is achieved (EC80) for ENL titrated against 63 nM H3K27cr (B) and H3K27cr titrated against 125 nM ENL (C). Based on these findings, 80 nM ENL and 40 nM H3K27cr was selected for subsequent experiments.*

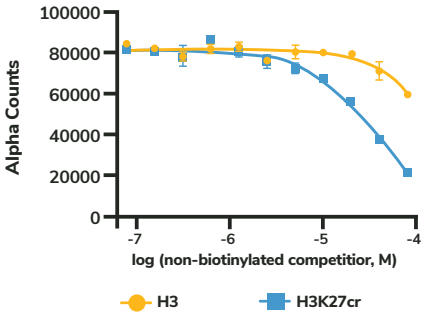

FIGURE 3: COMPETITION ASSAY. *6HIS-ENL (80 nM) was pre-incubated with the indicated non-biotinylated histone competitor peptides (10 point dose-response in 1:2 titrations) for 15 minutes. Biotin-H3K27cr (40 nM) was added for an additional 30 minutes. One hour after the addition of Donor and Acceptor beads, the Alpha Signal was read. Competition with the non-biotinylated H3K27cr peptide inhibited the Alpha signal in the low-to-mid micromolar range, consistent with the binding affinity of ENL to acyl-modified histones [5].*

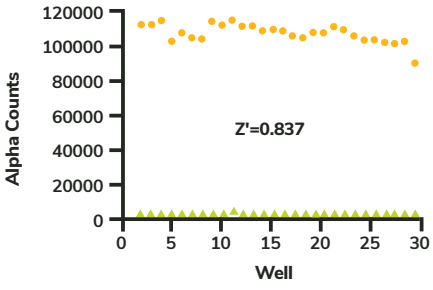

FIGURE 4: Z' FACTOR DETERMINATION. *6HIS-ENL (80 nM) was incubated with Biotin-unmodified H3 peptide (40 nM) as a negative control. Incubation with Biotin-H3K27cr (40 nM) determined the maximum signal. A Z' factor of 0.837 was calculated as described [6], indicating readiness for high throughput screening (Z'>0.5).*

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