

Spot-Trap and Spot-Label for CRISPR'ed Spot-Tag

The Spot-System can be applied for capture and detection of Spot-tagged proteins at endogenous expression levels

Introduction

ChromoTek's novel Spot-System is the first peptide-tag specific Nanobody for universal capture & detection applications. It comprises Spot-Tag®, an inert 12 amino acid peptide-tag, and the universal, rugged Spot-Nanobody that specifically binds to Spot-tagged proteins with a very high affinity. It can be used in multiple applications including immunoprecipitation (IP), Co-IP, affinity purification, immunofluorescence, and Western blotting.

Frequently, tagged proteins are analyzed from ectopically introduced overexpression constructs. However, also tagged proteins expressed at endogenous level under the control of endogenous promoters are of immanent interest to researchers. Technologies like CRISPR/Cas9 are popular tools to generate these. Here, the performance of the novel Spot-Tag and Spot-Nanobody is evaluated, when the Spot-Tag is introduced in-frame with a protein coding sequence into a genome. Hence, we generated the HeLa_LMNA-EGFP-Spot cell line. In this cell line, both Spot-Tag and EGFP were introduced into the LaminA/C locus after the start codon of the first exon of the LMNA gene using the CRISPR/Cas9 technology. In the resulting stable cell line, nuclear lamina contains Lamins A and C fused to both Spot-Tag and EGFP.

Materials and Methods

Immunoprecipitations of Spot-EGFP-LaminA/C were conducted using Spot-Trap and GFP-Trap according to the manufacturer's protocols. Immunostaining was done using Spot-Label according to the manufacturer's protocol (see www.chromotek.com). HeLa cell line expressing Spot-EGFP-LaminA/C fusion protein from LMNA locus (HeLa_LMNA-EGFP-Spot) was used at passages p3-p8. Untransfected HeLa cells were used as negative controls.

Results

1. Immunoprecipitation of Spot-EGFP-tagged-LaminA/Cat endogenous level using Spot-Trap

Spot-EGFP-LaminA/C fusion protein from lysed HeLa_LMNA-EGFP-Spot cells was immunoprecipitated using Spot-Trap® coupled to agarose beads (product code: eta). As control, Spot-EGFP-LaminA/C fusion protein was immunoprecipitated using GFP-Trap® coupled to agarose beads (product code: gta). Western blot analysis of the pull-down fractions was done showing: input (In, 5%), flow-through (FT, 5%), and bound fractions (B, 100%) (**Figure 1**). Spot-EGFP-LaminA/C were detected with rabbit polyclonal anti-GFP antibodies (product code: PABG1). Both, Spot-Trap and GFP-Trap successfully immunoprecipitated Spot-EGFP-LaminA/C fusion proteins at equal performance according to the Western blot.

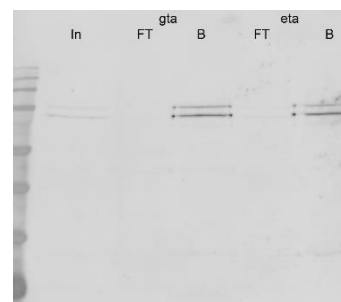


Figure 1: Western blot analysis of the pull-down fractions. Separate bands for LaminA and LaminC are detected.

Applications Note: Spot-Trap and Spot-Label for CRISPR'ed Spot-tagged proteins

2. Immunostaining of Spot-EGFP-tagged-LaminA/C at endogenous level using Spot-Label

Immunofluorescence with Spot-Label_ATTO594 (product code: eba594, 1:1,000) was performed on PFA-fixed and Triton-permeabilized HeLa_LMNA-EGFP-Spot cells (**Figure 2**). EGFP signal (green, left) and Spot-Tag immunostaining (red, right) from the endogenous-level expressed Spot-EGFP-LaminA/C fusion proteins co-localize at the nuclear lamina, as expected. As expected, the strength of both EGFP and Spot-Label signals is low due to the relatively weak target expression at endogenous level.

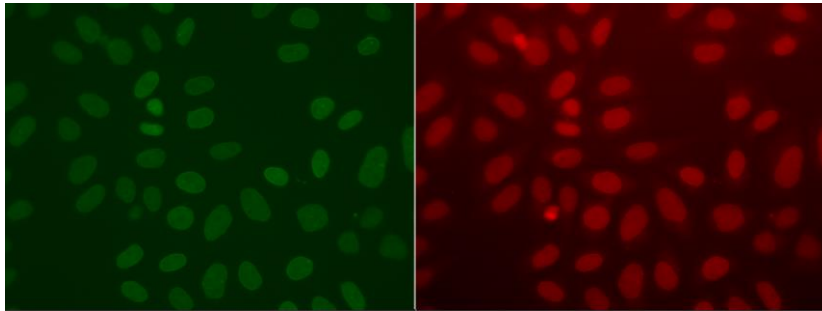


Figure 2: Epifluorescence images acquired with Leica microscope, 20X objective. EGFP signal (green, left) and Spot-Tag immunostaining (red, right)

Conclusion

ChromoTek's novel Spot-System can be used for immunoprecipitation and immunostaining of both overexpressed Spot-tagged protein constructs and Spot-tagged proteins that are expressed at physiological or endogenous level. The protocols provided with Spot-Trap and Spot-Label® are well applicable for these experiments, with the following recommendations for endogenous-level expressed Spot-tagged proteins:

- For Western blot analysis of the results of the immunoprecipitation using Spot-Trap, it is recommended to increase the amount of loaded fractions: e.g. 5% of input and flow-through, and 100% of bound fraction.
- For microscopy of the immunostainings with Spot-Label, it is recommended to use longer exposure times (exceeding 1 s) to acquire stronger signals.

References

<https://www.chromotek.com/products/spot/>

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