Recombinant Antibody-NanoLuc Fusions for Immunoassays

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1. Recombinant Antibody-NanoLuc Fusion

**Recombinant Antibody NanoLuc**

- NanoLuc is cloned with antibody heavy chain, expressed in CHO cells and purified.
- NanoLuc is an extremely bright and small (19kDa) bioluminescent protein.

**Attributes of Recombinant Antibody-NanoLuc**

- NanoLuc is site-specifically fused to the C-terminus of the heavy chain of the antibodies.
- Unlike chemical labeling, recombinant method results in homogeneous labeling with NanoLuc to antibody ratio = 2.
- NLuc is attached away from antigen binding site resulting in no impact on antibody-antigen binding.
- Lot-to-lot reproducibility prevents expensive and time-consuming revalidation of assays.
- Proof-of-concept: We made recombinant NanoLuc fusion of Trastuzumab (Herceptin) and Cetuximab (Erbitux) and show homogeneous labeling of antibodies and several applications.

3. Recombinant Antibody-NanoLuc Conjugates

**Non-reducing SDS-PAGE**

- NanoLuc was site-specifically fused to C-terminus of the heavy chain.
- Antibodies have 2 NanoLuc per antibody.
- Homogeneous population means batch-to-batch reproducibility.

Reducing SDS-PAGE

- NanoLuc is fused to the heavy chain of the antibodies.

4. Recombinant Antibody-NanoLuc Binding Activity

**ELISA Format**

In an ELISA assay, binding affinity of NanoLuc fused antibody is similar to that of native antibody.

5. One Step Pharmacokinetics Experiment

- Pharmacokinetics of antibody drugs is a critical parameter that determines the dosing and efficacy of the drug.
- Two-step ELISAs are commonly used to determine the concentration of drug in the serum.
- We optimized a single step immunoassay using Antibody-NanoLuc conjugate.

Site-specific recombinant antibody drugs fused to NanoLuc offers a single step competition ELISA protocol for quantifying drugs during pharmacokinetics experiment.

8. One Step Assay for Detection of Anti Drug Antibody (ADA)

- Higher concentration of ADA results in loss of signal.
- NanoLuc Antibody fusion allow a single step ADA detection assays directly from serum sample.

9. Summary

1. Recombinant antibody NanoLuc fusions have several advantages including:
   - Site specific attachment of NanoLuc reporter away from the antigen binding site.
   - Well defined ratio of two NanoLuc per antibody.
   - Small and bright reporter results in sensitive assays.
   - Batch to batch reproducibility of reagent.

2. Applications:
   - Ligand binding assay for Pharmacokinetics.
   - Anti drug antibody assays.
   - ELISAs for analyte detection.

3. We have also developed novel chemical conjugation method for oriented and efficient attachment of NanoLuc to the proteins and antibodies.