

Increased detection of HER dimer and downstream signaling proteins utilizing the VeraTag technology with dextran modified antibodies



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Abstract

Background. The VeraTag assay is a novel, fluorescent-based technology that quantifies functional protein-protein interactions and protein expression. We have applied the VeraTag technology to identify potential breast cancer markers that can be used to predict response to therapies targeted to the HER signaling pathways. The sensitivity of traditional fluorescent immunoassays is often limited by the dye to antibody ratio. Increasing this ratio often has a negative outcome on assay performance due to dye quenching or loss of antibody reactivity. We have developed a modified VeraTag immunoassay that utilizes a high VeraTag to antibody ratio to detect and measure two breast cancer markers; human epidermal growth factor receptor HER2-HER3 heterodimers and a downstream signaling molecule pAkt. In addition, a multiplexed assay format using a high VeraTag to antibody ratio was developed for the quantification of the inflammation markers TNF α , IFN γ , and IL-2. **Methods.** Commercially available antibodies were attached either directly to VeraTag reporters or indirectly through a 70kDa amino-dextran scaffold to increase the ratio of VeraTag reporters to antibody. The breast cancer cell line MCF7 was stimulated with heregulin to activate HER dimerization and Akt phosphorylation. Assays were developed for comparison of cell lysates from stimulated and unstimulated cells. Immunoassays for TNF α , IFN γ , and IL-2 were developed using purified antigens. Both directly attached VeraTag-antibody and VeraTag-dextran-antibody formats were evaluated and the performance of the two formats was compared. **Results.** The ratio of VeraTag reporters to antibody using a dextran scaffold increased 4- to 15-fold over the directly attached VeraTag-antibody. All assays showed a proportional increase in signal strength and sensitivity without a loss in the limit of detection. The modified antibodies were functional in both single-plex and multiplex immunoassay formats, with no detectable loss in performance due to multiplexing. **Conclusions.** These data demonstrate a useful approach to increasing immunoassay sensitivity for the detection and quantification of clinically relevant targets for breast cancer and inflammation. The increased sensitivity may allow for easier detection of low-level targets such as HER dimers and signaling proteins, possibly at an earlier stage of the disease.

Methods

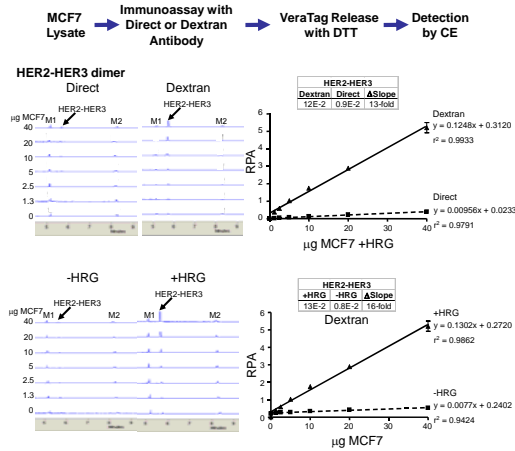
Direct conjugation of VeraTag to antibody



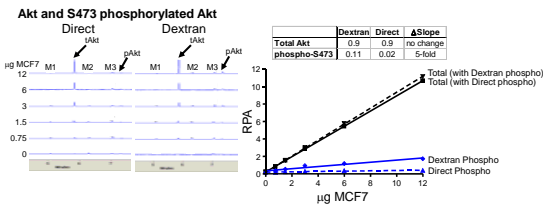
Conjugation of VeraTag to antibody through dextran

- VeraTag succinimidyl ester + 70kDa amino-dextran + SMCC \rightarrow VeraTag-Dextran-Maleimide
- SATP + Antibody \rightarrow HS-Antibody
- VeraTag-Dextran-Maleimide + HS-Antibody \rightarrow VeraTag-Dextran-Antibody

Results - Lysate

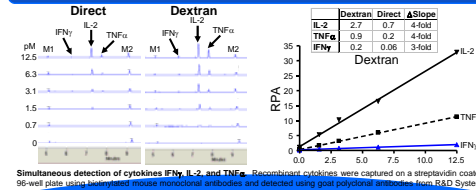


HER2-HER3 in heregulin stimulated MCF7 lysate. HER3 was captured on a streptavidin coated 96-well plate using biotinylated HER3 mouse monoclonal antibody 8A43 (Santa Cruz cat# sc2985). HER2-HER3 was detected using HER2 mouse monoclonal antibody N12 (Lab Vision cat# MS-301, Ab4). The HER2 antibody was either directly conjugated to 2 VeraTags/antibody or conjugated through dextran to 18 VeraTags/antibody.

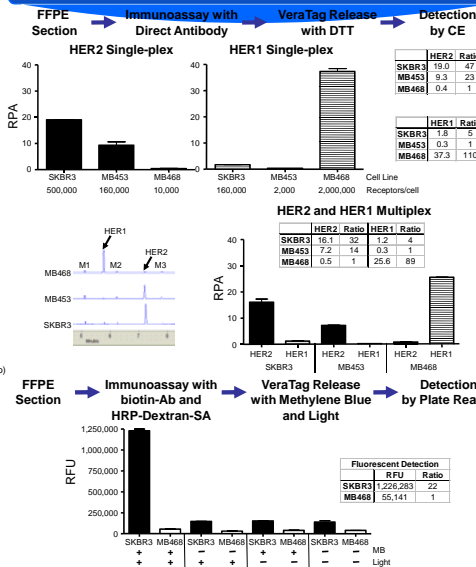


Simultaneous detection of phosphorylated (S473) and total Akt in heregulin stimulated MCF7 lysate. Akt was captured on a streptavidin coated 96-well plate using biotinylated Akt rabbit polyclonal antibody (R&D Systems cat# AF1775). Total Akt was detected using the same rabbit polyclonal antibody (R&D Systems cat# AF1775) directly conjugated to 2 VeraTags/antibody. Akt phosphorylated at S473 was detected with a rabbit polyclonal antibody (R&D Systems cat# AF887), either directly conjugated to 3 VeraTags/antibody or conjugated through dextran to 12 VeraTags/antibody.

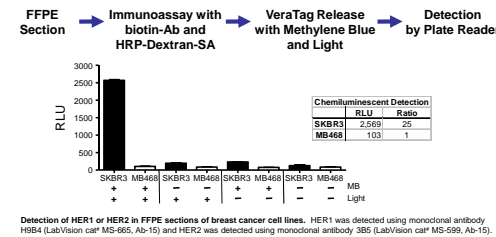
Results - Lysate



Results - FFPE



Results - FFPE



Summary

We have demonstrated enhancements to VeraTag assays resulting in increased signal intensity and sensitivity.

Lysate:

- Human epidermal growth factor receptor HER2-HER3 heterodimer and downstream signaling proteins Akt and S473 phosphorylated Akt.
- A multiplexed assay for the quantification of inflammation markers TNF α , IFN γ , and IL-2 was established using the high VeraTag-antibody ratio format.
- A 4- to 15-fold increase in VeraTag reports was attached to reporter antibodies.
- All assays showed a proportional increase in signal intensity and sensitivity with no loss to the limit of detection.
- The assays are functional in both single-plex and multiplex formats.
- No detectable loss in performance due to multiplexing.

FFPE:

- Quantitative measurement of human epidermal growth factor receptors HER1 and HER2 in FFPE breast cancer cell lines.
- HER1 and HER2 measurable in single-plex or multiplex format.
- Similar HER1 and HER2 dynamic range when multiplexed.
- Antibodies attached to releasable HRP allowed for the detection of HER2 on a standard plate reader
- Equivalent HER2 dynamic range with either fluorescent or chemiluminescent detection.
- Plate reader based assays being developed for the detection of HER dimers and other signaling proteins.

Acknowledgements

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