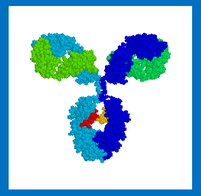

HTRF - A Beneficial Tool for Lead Optimization of Biotherapeutics

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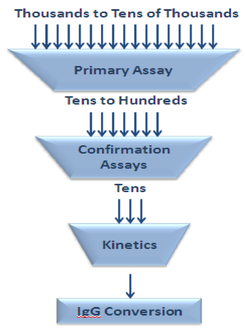
Abstract

The drug discovery process requires substantial screening efforts to identify suitable hits. These hits must then be optimized to generate leads with ideal properties. This optimization process relies heavily on ELISA format assays to select the top candidates. It is well known that, HTRF is a highly sensitive, robust, "mix and read" technology, frequently used for drug target studies in high-throughput screening. In an effort to improve our lead optimization process, we compared HTRF assays to our typical ELISA screening assays including: primary, confirmation, quantitative, competitive and whole cell assays. In the assay formats tested, data generated by HTRF was as good or of better quality than that by ELISA, with the added benefit of saving time by increasing the throughput of our assays by 4-fold. HTRF is a beneficial tool for a range of assays utilized in the lead optimization process.

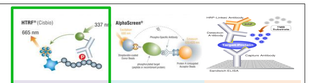
ELISA-Based Screening Paradigm



- Variants are generated to optimize the properties of the lead molecule
- Binding properties of variants are benchmarked against the lead molecule
- ELISA screening process is time consuming – assay alternatives needed

HTRF vs Other Screening Formats

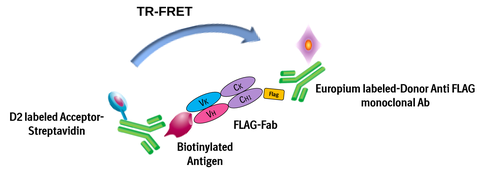
3 Screening Formats Were Assessed by 11 Parameters



	HTRF	Alpha Screen	ELISA
Detection Format	TR-FRET	Luminescence Proximity	Absorbance
Assay Format	Homogeneous	Homogeneous/Bead	Heterogeneous
No. of Dispensing Steps	2	2	7 + Washes
Assay Time	1hr	1hr	7hrs
Throughput	High	High	Medium
Miniturization (~384, ~1526)	Yes	Yes	No
Sensitivity to Light	No	Yes	Yes
Sensitivity to Temperature	No	Yes	Yes
Potential for False Positives	No	Yes	Yes
Repetitive Reads	Yes	No	No
Z'	Excellent	Good	Good

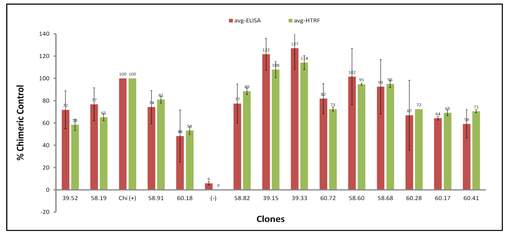
➢HTRF ranked highest overall

Primary and Confirmatory Screening Assays



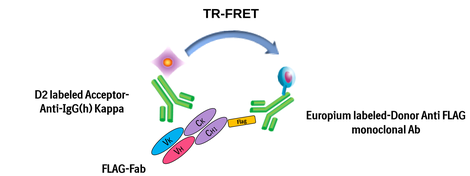
Fab binding to antigen will result in a FRET signal

Screening Data Quality of HTRF vs ELISA



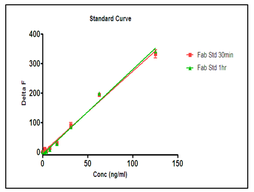
➢Fabs are ranked by percent of chimeric binding interaction
➢Binding values are equivalent between HTRF and ELISA
➢A range of expression does not influence binding (data not shown)
➢Day-to-day and sample lot deviation lower for HTRF over ELISA

Quantitation Assay



An intact Fab, with heavy and light chain, will result in a FRET signal

Quantitation Values are Obtained as a Tool for Other Assays

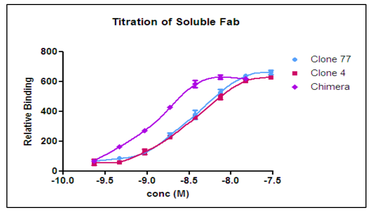


	Concentration µg/ml		
Variant ID	ELISA	HTRF	Δ HTRF/ELISA (%)
1	0.40	0.44	10
11	1.10	1.15	5
15	1.66	1.45	-13
14	2.04	2.20	8
9	2.53	2.25	-11
7	3.91	3.52	-10
6	4.40	3.87	-12
5	5.15	5.20	1
19	7.85	8.63	10
20	11.88	11.47	-3

➢Quantitation values are equivalent between HTRF and ELISA
➢HTRF allows a large dynamic range of 0.4 – 12µg/ml
➢Assay time reduced from 7hrs (ELISA) to 2hrs (HTRF)

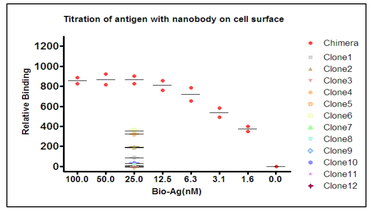
Titration Assays

Kinectics of Soluble Fab Variants



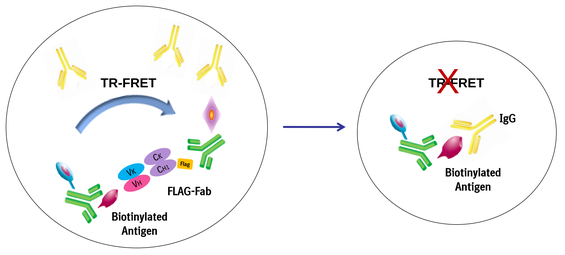
➢Titration assays enable distinction of on and off rates

Ranking of Variants Expressed on Cell Surface



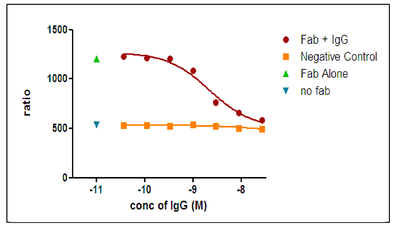
➢Binding to cell surface targets can be done with HTRF technology

Competition of Fab with IgG



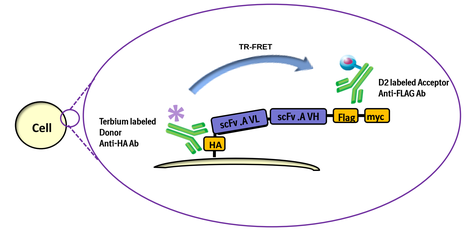
Optimized variants are benchmarked to lead molecule by direct competition

Competition of Fab Variant with Titrated IgG



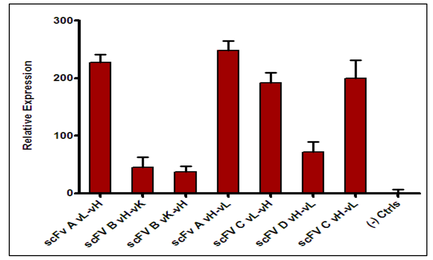
➢IC50's are easily generated by HTRF competition assays

Quantification of scFv's on Cell Surface



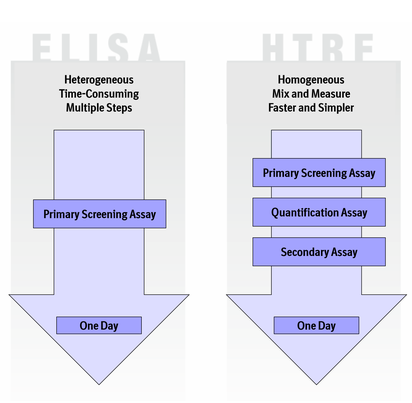
Anti-HA Tb and Anti-FLAG d2, mixed with cells, generate FRET signal

Expression Levels of Cell Presented Variants



➢ScFv's are shown to express at different levels on the cell surface

Summary



➢HTRF has been adapted for multiple applications in lead optimization
➢HTRF provides the ability to introduce cellular assays
➢Data quality is equivalent to ELISA
➢Reproducibility between assays is consistently observed
➢Time savings leads to increased data output