

APPLICATION NOTE

HTRF profiling services on SpectraMax iD5 Multi-Mode Microplate Reader

Natalia Castro | Assay Development Scientist | Cisbio
Arturo Gonzalez Moya | Custom Solutions Manager | Cisbio
Cathy Olsen, PhD | Sr. Applications Scientist | Molecular Devices

Introduction

Cisbio's Custom Solutions Laboratory team has worked in collaboration with Molecular Devices to generate relevant data promoting the expert profiling service activity completed by Cisbio US utilizing current Molecular Devices readers.

The SpectraMax® iD5 Multi-Mode Microplate Reader is HTRF-certified, with an enhanced TRF detection module and filters optimized for HTRF detection. Preconfigured HTRF protocols in SoftMax® Pro Software 7.0.3 and higher include optimized reader settings, as well as data analysis, for faster results.

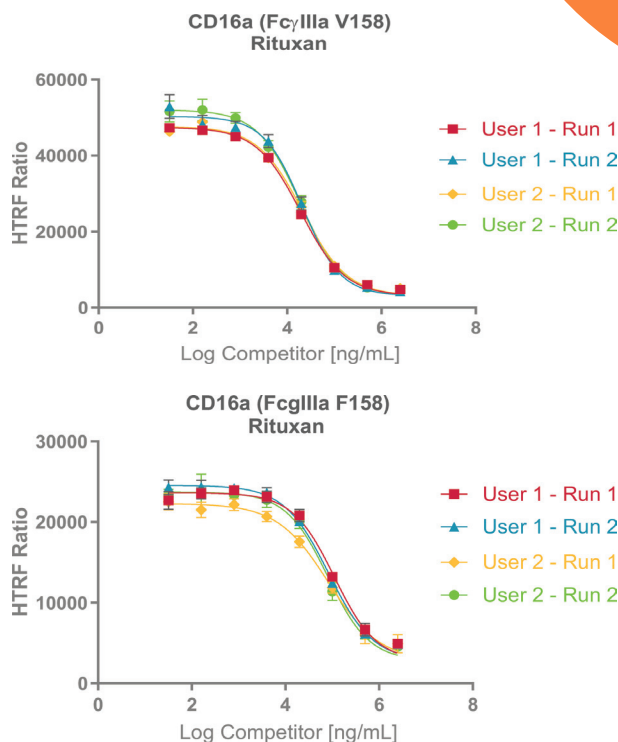
Experimental details

A profiling service was completed using three commercial drugs all on Cisbio's Fcγ receptor binding assays and FcRn. The SpectraMax iD5 reader was used for measurement of the results. For each drug/antibody used, Cisbio established:

- Dose-response curves in triplicate with eight (8) concentrations
- Control 1: Labeled cells expressing the receptor of interest only (min)
- Control 2: Labeled cells expressing the receptor of interest and IgG-red only (max)

Compound dose response: 8-point dose response curve of serially diluted compound performed in triplicate.

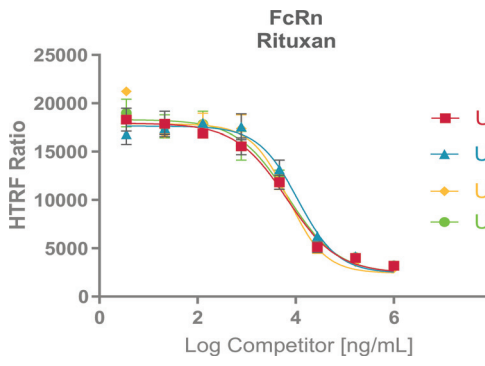
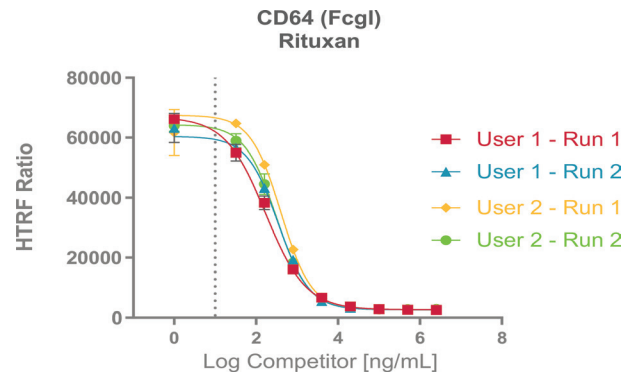
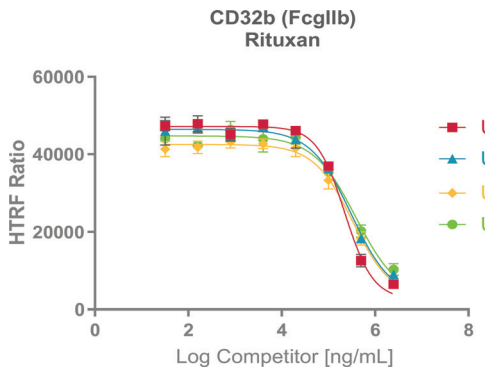
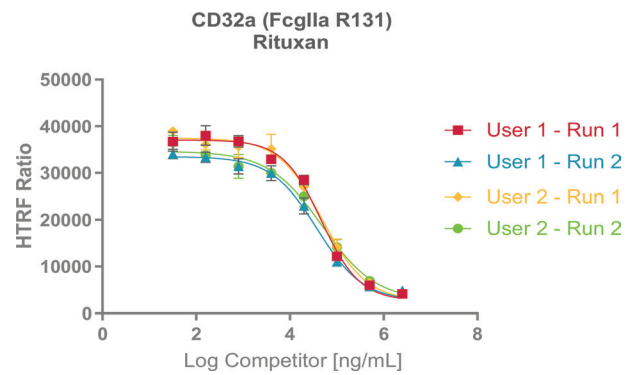
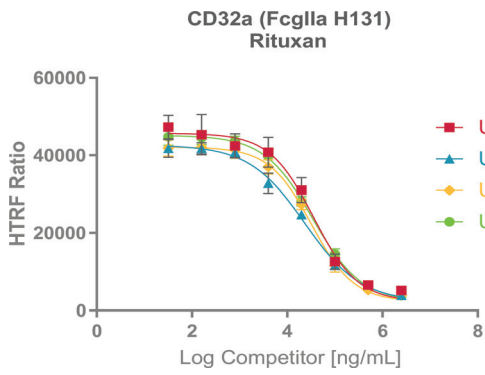
The following graphs contain the dose responses from two (2) users on two (2) different days. Data includes one single sample in all Fcγ receptors and FcRn assay (SpectraMax iD5 reader), with no outliers, bottom constrained and concentration [ng/mL].



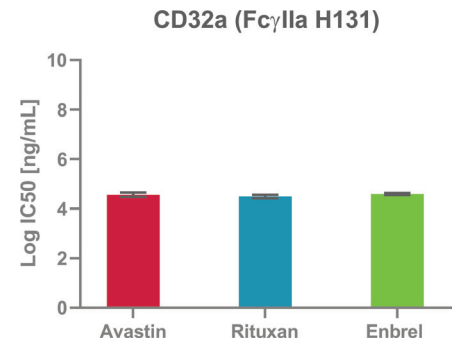
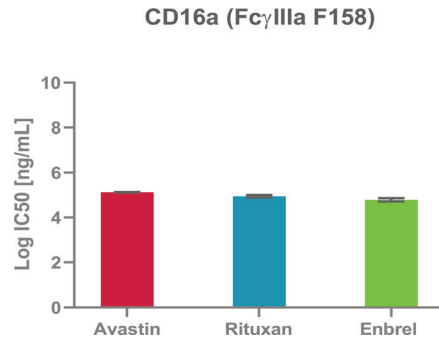
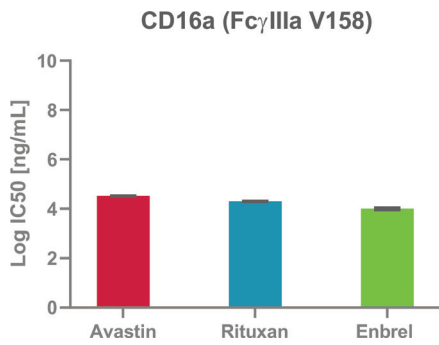
Benefits

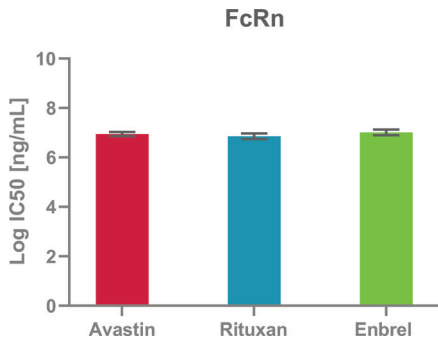
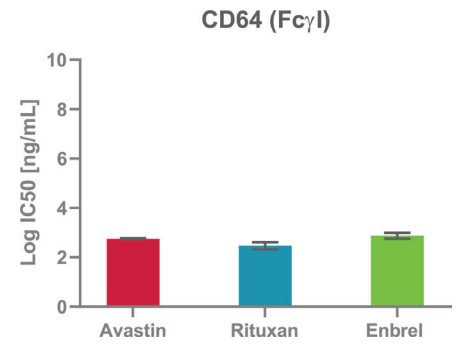
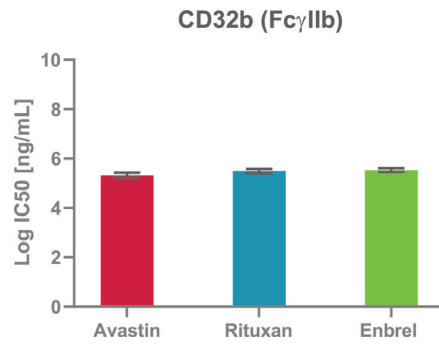
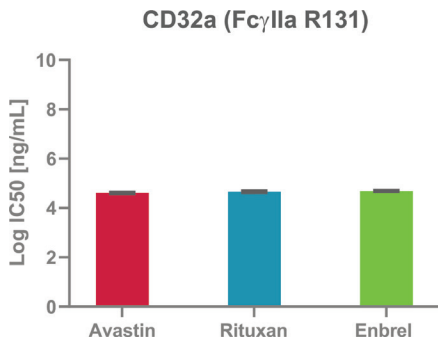
- Obtain consistent compound profiling results with the HTRF-optimized SpectraMax iD5 Multi-Mode Microplate Reader
- Analyze data quickly with preconfigured protocols in SoftMax Pro Software
- Access a wide range of acclaimed HTRF assays and services with Cisbio's US Custom Solutions Laboratory team

Sample #	Biosimilar	Trade Name	Mechanism of Action
1	Bevacizumab	Avastin	Anti-VEGFA mAb
2	Rituximab	Rituxan	CD20 (ADCC)
3	Etanercept	Enbrel	Anti-TNFα mAb



The following graphs show Log IC₅₀ and CV% values for all samples. Samples were run over multiple days with multiple users with readings from Molecular Devices SpectraMax iD5 Reader.





The following tables represent the Log IC₅₀ [ng/mL] and CV% raw values from seven (7) receptors, two (2) users, and readings from the SpectraMax iD5 reader.

CD16a (FcγIIa V158)			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	4.516	4.285	4.29
User 1 – Run 2	4.544	4.317	4.235
User 2 – Run 1	4.521	4.327	4.174
User 2 – Run 2	4.528	4.279	4.158
AVG	4.527	4.302	4.214
STDV	0.011	0.02	0.052
CV%	0.2%	0.5%	1.2%

CD16a (FcγIIa F158)			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	5.137	5.045	4.875
User 1 – Run 2	5.112	4.922	4.848
User 2 – Run 1	5.13	4.919	4.748
User 2 – Run 2	5.134	4.906	4.685
AVG	5.128	4.948	4.789
STDV	0.01	0.056	0.076
CV%	0.2%	1.1%	1.6%

CD32a (FcγIIa H131)			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	4.445	4.556	4.598
User 1 – Run 2	4.606	4.373	4.647
User 2 – Run 1	4.679	4.511	4.528
User 2 – Run 2	4.524	4.536	4.609
AVG	4.564	4.494	4.596
STDV	0.088	0.072	0.043
CV%	1.9%	1.6%	.09%

CD32a (FcγIIa R131)			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	4.57	4.671	4.654
User 1 – Run 2	4.576	4.58	4.691
User 2 – Run 1	4.649	4.693	4.742
User 2 – Run 2	4.656	4.695	4.662
AVG	4.613	4.660	4.687
STDV	0.04	0.047	0.034
CV%	0.9%	1%	0.7%

CD32b (FcγIIb)			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	5.184	5.353	5.499
User 1 – Run 2	5.258	5.494	5.442
User 2 – Run 1	5.389	5.526	5.545
User 2 – Run 2	5.447	5.593	5.646
AVG	5.32	5.492	5.533
STDV	0.104	0.088	0.075
CV%	2%	1.6%	1.4%

CD64 (FcγI)			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	2.751	2.23	2.937
User 1 – Run 2	2.744	2.537	2.824
User 2 – Run 1	2.778	2.605	3.028
User 2 – Run 2	2.759	2.494	2.712
AVG	2.758	2.467	2.875
STDV	0.0127	0.1422	0.1188
CV%	0.5%	5.8%	4.1%

FcRn			
	Avastin (Bevacizumab)	Rituxan (Rituximab)	Enbrel (Etanercept)
User 1 – Run 1	6.977	6.812	6.958
User 1 – Run 2	7.022	7.046	7.019
User 2 – Run 1	6.976	6.742	6.875
User 2 – Run 2	6.812	6.833	7.191
AVG	6.947	6.858	7.011
STDV	0.08	0.114	0.116
CV%	1.2%	1.7%	1.7%

Conclusion

Due to the collaboration with Molecular Devices and the SpectraMax iD5 Multi-Mode Microplate Reader, Cisbio can now offer custom HTRF profiling services as a reliable, fast and reproducible approach to drug discovery using technology suitable for antibody affinity and the potential of biosimilarity studies using relevant drugs available on the market.

Contact Us

Phone: [+1.800.635.5577](tel:+18006355577)
 Web: www.moleculardevices.com
 Email: info@moldev.com
 Check our website for a current listing of worldwide distributors.