

Summary of Human IFNg Assay:

Assay	Human IFNg
E file	E584
Developer	Saili Yi
Date Summary	4-14-2010

- ✓ The assay detects human IFNg. hIFNg from R&D system was used as calibrator (1.2-1000 Pg/ml) in Theranos OC assay.

- ✓ Finished Experiment as following:

1	MTP screen: 12 pairs of antibodies have been screened.
2	Cross reactivity: no cross reaction with IL1b, IL4, IL6 and TNFa
4	Interference: IL1b, IL4, IL6 and TNFa do not interfere in this assay.
6	Theranos screen: 4 pair's antibodies were screened in whole blood and plasma on Edison. Final antibody pair was selected.
10	Capture titration: Cab 5, 10 and 20 ug/ml were compared. Cab 5 ug/ml was used for further optimization.
11	Detection titration: Dab 25, 50, 100 and 200 ng/ml were compared. Dab 50 ng/ml was selected for assay.
12	Detection Stabilizer: Stabilizyme and BioStab have been compared. Stabilizyme gave better result.
13	Effect of Buffer: Five different buffers (Theranos blocking buffer, protein free blocking buffer, Starting blocking buffer, Superblock and blockiner Casein) for coat have been compared. Theranos assay buffer produces best result.
14	Incubation time: coincubation was used to increase the sensitivity
15	Edison protocol optimization: 1x post sample wash was selected for further optimization.
16	% CV : Ave. Intra % CV 14%, total % CV 13%

Partially complete

18	Calibrator comparison	Based on the result using IFNg from NIBSC, the IFNg from R&D system is overestimate.
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- ✓ Uncomplete

8	Plasma Screen	
9	Whole Blood Screen	
17	Precision tests	
18	Calibrator comparison	
19	Dilution linearity	
20	Whole blood spike recovery	
21	Hematocrit effect	
22	Plasma spike recovery	
23	LLOQ/ULOQ	
24	Selectivity	
26	Matrix effects	OC in hemolysed or lipemic samples has been test.
27	Extended range	
28	Clinical samples	

Assay Reagents:

A. Capture Antibody

Vendor	BD
Catalog #	551221
Current lot #	NIB42
Type	m IgG1,
Immunogen	Human IgG
Specificity	human
Stock Conc.	1 mg/mL
Working conc.	5 ug/mL in assay buffer
Location	4C at back of Lab.
Availability	12-18-10 conjugated , 40 ul 1-25-10 conjugated 350 ul No un-conjugated Cab in Lab.

B. Detection Antibody

Vendor	abcam
Catalog #	Ab18654
Current lot #	678004
Type	mIgG1
Immunogen	Purified recombinant full length protein (Human).
Specificity	human
Stock Conc.	1 mg/ml
Working conc.	100 ng/mL in stabilzyme
Location	4C at back of Lab.
Availability	40 ul 250 ng/ml, need to order new Dab

C. Current used Analyte: human IFNg

Vendor	R&D system
Catalog #	285-IF/CF
Current lot #	EA4009041
Type	Synthesis peptide
Stock Conc.	0.263 mg/ml
Storage	Undiluted stock at 4C, 10 ug/ml stock at -20C,
Availability	10 ug/ml stock at -20C, enough for half year It is suggested that order new analyte every 6 month. New analyte is needed to order.

D. Analyte from other sources: human IFNg

Vendor	NIBSC	BD
Catalog #	87/586	554617
Current lot #	No	54767
Type	IFNg protein	IFNg protein
Stock Conc.	12.5 ng per ampoule	500 ug/ml

Storage	-20C	-20C
Availability	3 ampoules	50 ug

E. Analyte Diluent

Composition	50 mM TBS, 3% BSA, 0.05% Thimerosal, pH 8.0
Storage	4C,

F. Clinical sample: ProMed, 1 mlX20, -20C

G. hIFNg ELISA kit

Company	cat#	range	sensitivity	Normal level	Price (kit)	Availability
abcam	ab46048	0.78 - 25 pg/ml	< 0.69	0.7-5.8 Pg/ml	\$460	2 kit, store at 4C
BD	550612	4.7-300 Pg/ml	1 pg/ml	<4 Pg/ml	550/2	0.5 kit, store at 4C

✓ Detection antibody Dilution

Dab - Alk Phos Stocks	Stock ug/mL	Final ug/mL	Vol stock uL	Diluent uL	Total Vol. uL	Diluent
Solution 1	500	10	20	980	1000	Stabilzyme/SatbilizGuard
Solution 2	10	0.1	10	990	1000	Stabilzyme/SatbilizGuard

Protocol for assay on the Edisons

Protocol features: 10/10 co-incubation assay protocol

	Protocol name	Svn #	Sample dilutions	Tip positions
1	Generic_ND_D_10_10 test;	3315	3X	tips 1,2

Protocol for assay on 384 well

10/10/10 protocol for 384 well