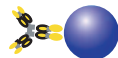


Technical Note 3: Nanoplex™ Direct No-wash Assay

Nanoplex™ Direct is a homogeneous assay format rivalling conventional ELISAs while offering several advantages including

- Simple procedure using a one-step, no-wash assay format
- High sensitivity, on par with heterogeneous, amplified systems
- Leverages the performance of surface-enhanced Raman scattering (SERS)
- Near IR detection allows for data collection in whole blood, serum and plasma backgrounds
- Multiplexing capability of the technology allows for quantitation of multiple analytes in a single reaction
- Benchtop reader and proprietary software for data analysis

Procedure



Nanoplex™ biotag conjugated to antibody 1



Magnetic bead conjugated to antibody 2

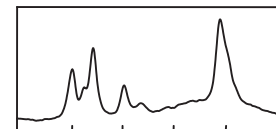


Analyte

Step 1: Add analyte and incubate

Step 2: Magnetic pull down to pellet

Step 3: Acquire spectrum



Assay Time 30 min

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Advantages of Nanoplex™ Direct

Detection of TNF- α comparing ELISA and Nanoplex™ Direct workflow

	ELISA*	NANOPLEX™ DIRECT
Total Assay Time	5 hours	< 1 hour
Number of wash steps	3	0
Amount of TNF- α antibody	400 ng/well	450 ng/tube
Capture	7 ng/well	8 ng/tube
Detection		
Detection Limit	2 pg/mL	3 pg/mL

* Reported values

Precision in Biological Samples

Example of cTnI and CKMB detection in serum samples (Bio-Rad Liquicheck Cardiac Markers Plus Controls) in a multiplexed assay

	COMMERICAL ANALYZER*	NANOPLEX™ DIRECT	COMMERICAL ANALYSER*	NANOPLEX™ DIRECT
BioRad Controls	cTnI Clinical Analyzer Range ng/ml	cTnI 2-plex Nanoplex™ Direct ng/ml(**)	CKMB Clinical Analyzer Range ng/ml	CKMB 2-plex Nanoplex™ Direct ng/ml(**)
Level 1	0.4 – 2.2	2 (14)	2 – 5	4 (8)
Level 2	3 – 20	13 (8)	10 – 18	17 (14)
Level 3	9 – 50	28 (4)	27 -81	40 (12)

* Values determined on 20 commercial clinical analyzers

** CV %, 6 repetitions

Multiplexed Technology

- Quantitation of multiple analytes in a single reaction
- Assays perform independently of each other

