



Cardiac Biomarker Assays

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Meso Scale Discovery (MSD) has developed immunoassays for the quantitative measurement of serum biomarkers related to human cardiac injury and disease: C-Reactive Protein (CRP), Myeloperoxidase (MPO), Troponin-T, Troponin-I, CKMB, and Myoglobin.

The assays are performed on the MSD® MULTI-ARRAY™ platform, using MSD's MULTI-ARRAY 96-well plates and SECTOR™ instrumentation. All assays are formatted as sandwich immunoassays: capture antibodies are pre-coated onto the plates, and electrochemiluminescent-labeled detection antibodies are supplied. Assay protocols are simple: they require few wash steps, use incubation steps of either one or two hours, have a rapid read time of approximately one minute per plate, and are validated for serum and EDTA/heparin plasma samples. Certain combinations of assays are also available in multiplex format. For example, Troponin-I, CKMB and Myoglobin are available as a triplex in 96-well 4-spot plates, allowing simultaneous measurement of all three markers from a single sample volume of 25 μ L.

Assay ranges are 0.01 – 200 μ g/mL for CRP, 0.6 – 1000 ng/mL for MPO, 0.003 – 25 ng/mL for Troponin-T, 0.01 – 80 ng/mL for Troponin-I, 0.15 – 400 ng/mL for CKMB, and 19 – 3000 ng/mL for Myoglobin. All assays are highly specific and linear over normal clinical ranges.

Background and Assay Format

- MSD cardiac assays are performed in MSD MULTI-ARRAY 96-well plates, and read on MSD SECTOR Instruments.
- MSD assays use electrochemiluminescence detection that allows for very sensitive measurements (e.g. detection limit of 3 pg/mL for Troponin-T).
- All assays are formatted as sandwich immunoassays, using monoclonal antibody pairs.
- Assay kits contain the following components:
 - Plates: MSD MULTI-ARRAY plates pre-coated with capture antibodies.
 - Detection antibody, pre-labeled with an electrochemiluminescent $\text{Ru}(\text{bpy})_3^{2+}$ compound.
 - Diluents: formatted for optimal sensitivity and precision, containing additives to eliminate HAMA and other interference.
 - Calibrators: calibration is performed with industry-accepted standards — a high concentration of calibrated analyte is supplied in each kit for generating a dilution curve.
- Multiplex assays utilize MULTI-SPOT® plates. For example, the Troponin-I/CKMB/Myoglobin triplex uses a 4-spot MULTI-SPOT plate which is pre-coated with the three capture antibodies on separate spots within each well.

Note: These assays are for research use only: not for use in diagnostic procedures.

Assay Protocols

Protocol for MPO and CRP Assays:

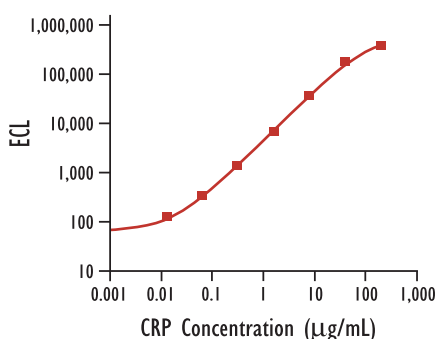
- Block MSD MULTI-ARRAY plate for 1 hour, wash
- Dilute sample 1:200 with assay diluent
- Add 40 μ L of assay diluent solution to each well
- Add 10 μ L of calibrator or diluted sample to each well
- Incubate with shaking for 60 minutes, wash
- Add 25 μ L of labeled antibody solution to each well
- Incubate with shaking for 60 minutes, wash
- Add MSD Read Buffer
- Read plate on MSD Reader

Protocol for Troponin-I, Troponin-T, CKMB and Myoglobin Assays:

- Begin with a MSD MULTI-ARRAY plate
- Add 25 μ L of labeled antibody solution to each well
- Add 25 μ L of sample or calibrators to each well
- Incubate with shaking for 60 minutes, wash
- Add MSD Read Buffer
- Read plate on MSD Reader

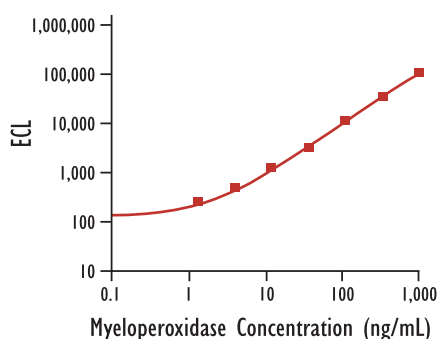
Assay Performances

CRP Calibration Curve



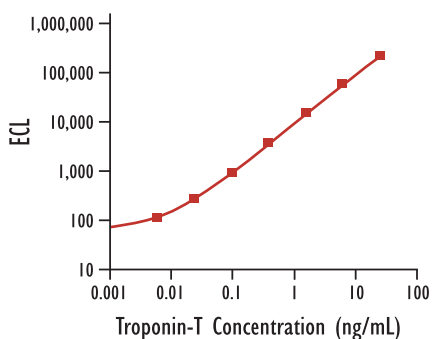
Sensitivity: 0.01 µg/mL
Dynamic Range: 0.01 to 200 µg/mL
Variability: less than 7% CV

Myeloperoxidase Calibration Curve



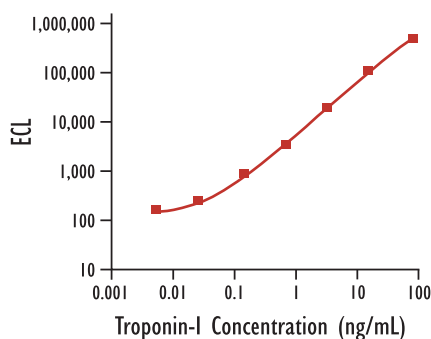
Sensitivity: 0.6 ng/mL
Dynamic Range: 0.6 to 1,000 ng/mL
Variability: less than 7% CV

Troponin-T Calibration Curve



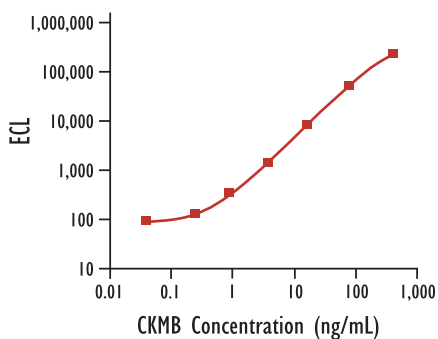
Sensitivity: 0.003 ng/mL
Dynamic Range: 0.003 to 25 ng/mL
Variability: less than 7% CV

Troponin-I Calibration Curve



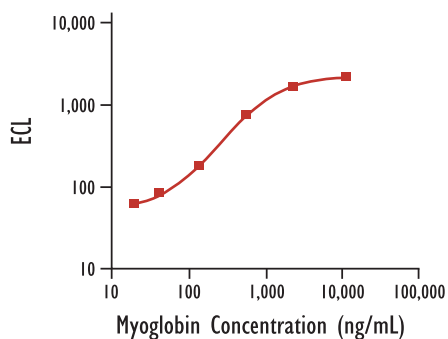
Sensitivity: 0.01 ng/mL
Dynamic Range: 0.01 to 80 ng/mL
Variability: less than 7% CV

CKMB Calibration Curve



Sensitivity: 0.15 ng/mL
Dynamic Range: 0.15 to 400 ng/mL
Variability: less than 8% CV

Myoglobin Calibration Curve



Sensitivity: 19 ng/mL
Dynamic Range: 19 to ~3,000 ng/mL
Variability: less than 10% CV

Performance Summary

Analyte	MSD Cardiac Assays		Clinical Analyzer and ELISA Assays		
	Analytical Sensitivity*	Linear Range	Analytical Sensitivity	Linear Range	System
CRP	0.01 µg/mL	200 µg/mL (no hook to 5,000 µg/mL)	0.1 µg/mL	No hook to 4,000 µg/mL	DCP Immulite® 2,000 hsCRP
MPO	0.6 ng/mL	1,000 ng/mL (no hook to 5,000 ng/mL)	1.5 ng/mL	50 ng/mL	Calbiochem ELISA
Troponin-T	0.003 ng/mL	25 ng/mL	0.01 ng/mL	25 ng/mL	Roche Elecsys®
Troponin-I	0.01 ng/mL	80 ng/mL	0.03 ng/mL	100 ng/mL	Beckmann Access®
CKMB	0.15 ng/mL	400 ng/mL	0.1 ng/mL	500 ng/mL	Roche Elecsys
Myoglobin	19 ng/mL	3,000 ng/mL	21 ng/mL	3,000 ng/mL	Roche Elecsys

* For MSD assays, analytical sensitivity = analyte concentration at 2.5 StdDev above background.

Dilution Linearity

Serum samples from different individuals containing detectable amounts of CRP, MPO, Troponin-T, Troponin-I, CKMB, or Myoglobin were diluted with the appropriate calibrator diluent from MSD cardiac assay kits. Recoveries were measured at three dilution levels – the average recovery at each dilution level is provided.

Analyte	Average Recovery at 1/2 Dilution Factor	Average Recovery at 1/4 Dilution Factor	Average Recovery at 1/8 Dilution Factor
CRP	97%	103%	107%
MPO	96%	101%	96%
Troponin-T	96%	98%	107%
Troponin-I	101%	104%	107%
CKMB	103%	101%	107%
Myoglobin	97%	103%	110%

Spike Recovery

Serum samples from different individuals were spiked with known quantities of CRP, MPO, Troponin-T, Troponin-I, CKMB, or Myoglobin at different levels and quantitated on MSD cardiac kits. Average recoveries for each assay are provided.

Analyte	Average Recovery
CRP	96%
MPO	100%
Troponin-T	102%
Troponin-I	97%
CKMB	105%
Myoglobin	108%

Expected Values

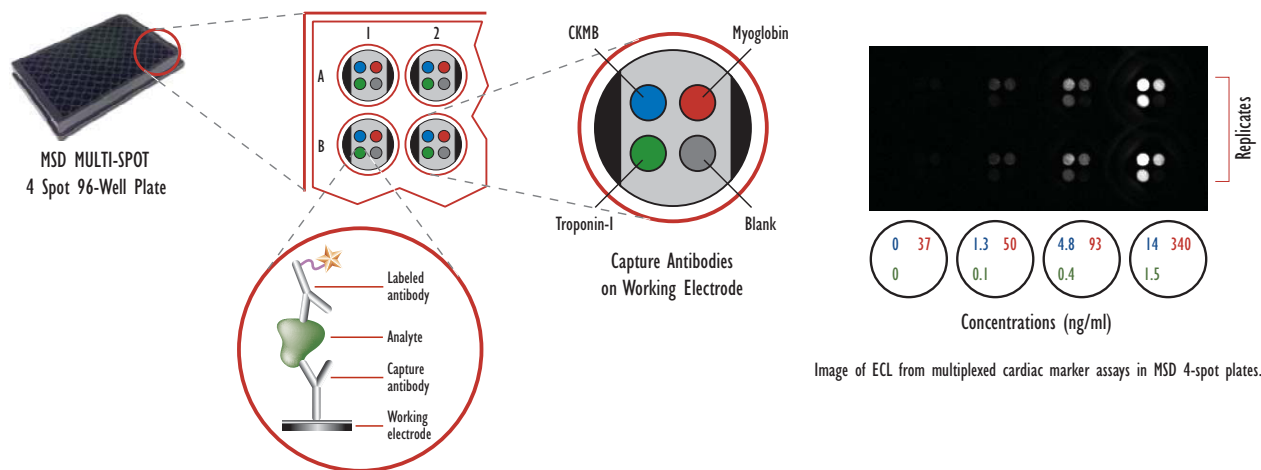
A reference range study was performed using serum samples from healthy donors, approximately half female and half male. Samples were measured with MSD cardiac assay kits to obtain the tabulated data.

Marker	N	Median	Mean	Range (95th Percentile)
CRP	20	2.3 µg/mL	4.5 µg/mL	0.07 - 19.1 µg/mL
MPO	20	55 ng/mL	64 ng/mL	21 - 149 ng/mL
Troponin-T	24	< 0.003 ng/mL	0.006 ng/mL	n/d - 0.030 ng/mL
Troponin-I	24	0.010 ng/mL	0.014 ng/mL	0.01 - 0.024 ng/mL
CKMB	24	1.44 ng/mL	1.54 ng/mL	0.7 - 2.9 ng/mL
Myoglobin	24	27.0 ng/mL	20.8 ng/mL	18.1 - 26.3 ng/mL

Multiplexing

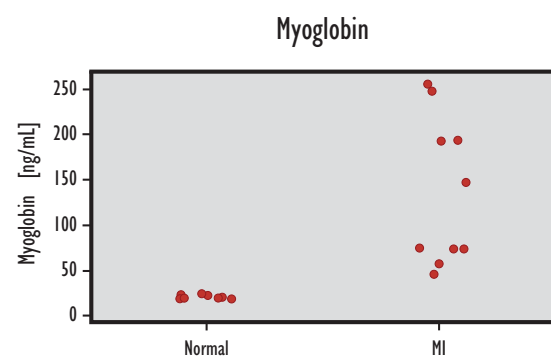
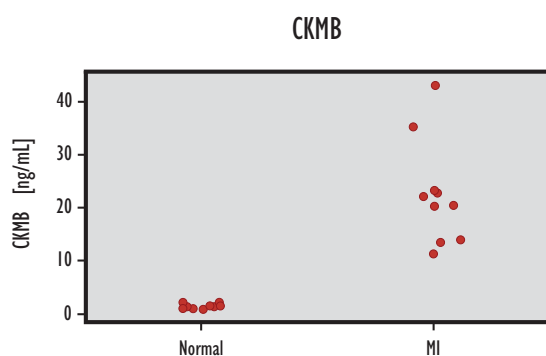
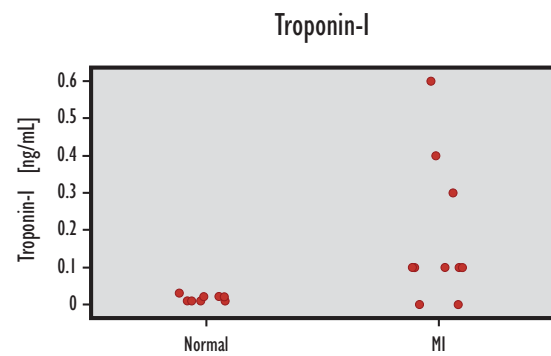
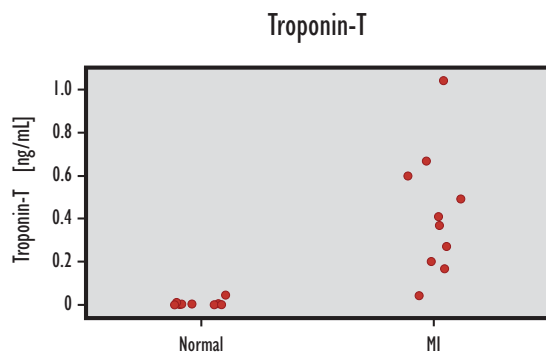
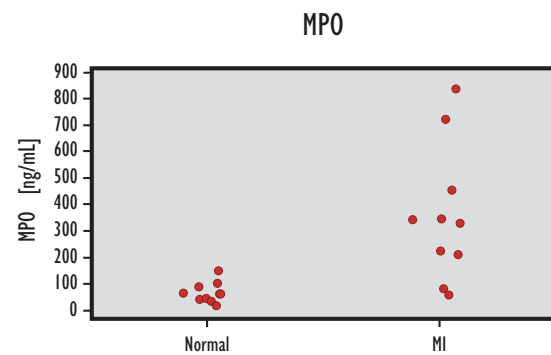
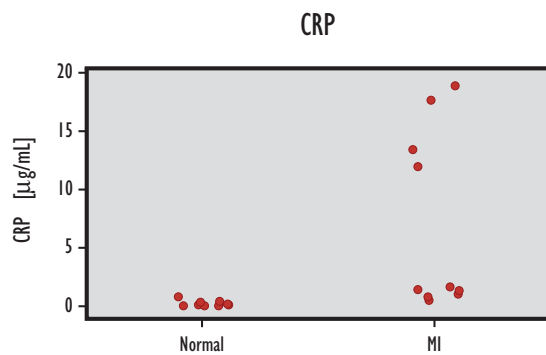
Certain MSD cardiac assays can be multiplexed, allowing simultaneous quantitation of multiple analytes in a single sample volume. Multiplexing saves effort, time, and sample.

Multiplex assays are formatted on MULTI-SPOT plates containing multiple capture zones within each well, each zone containing a different capture antibody. The Troponin-I/CKMB/Myoglobin triplex, formatted on a 4-spot plate, is shown as an example.



Clinical Sample Testing: Heart Attack Samples

To demonstrate the clinical utility of MSD cardiac assays, samples were obtained from ten patients within two days of suffering a myocardial infarction (MI). Each sample was measured on the MSD cardiac assay kits, along with ten samples from a non-diseased control group. Average concentrations for all markers increased for the MI group over the control group.



Conclusions

- Immunoassays for the human cardiac biomarkers CRP, MPO, Troponin-T, Troponin-I, CKMB, and Myoglobin have been demonstrated on MSD's MULTI-ARRAY platform.
- Assay kits are available that contain plates and all reagents required to perform the assays on human serum or plasma samples.
- Assays are rapid (1 or 2 hours), simple (minimal washes), use small sample volumes (<25 μ L), are very sensitive, and have wide dynamic ranges (>3 logs).
- Certain combinations of assays can be multiplexed, as demonstrated by the Troponin-I, CKMB, and Myoglobin triplex. Multiplexing allows simultaneous quantitation of multiple markers in a single sample volume.
- Assay performance is comparable or superior to clinical laboratory analyzers or ELISA kits.