

# Multiplexed Cardiac Marker Assays: CKMB, Myoglobin, and Troponin-I

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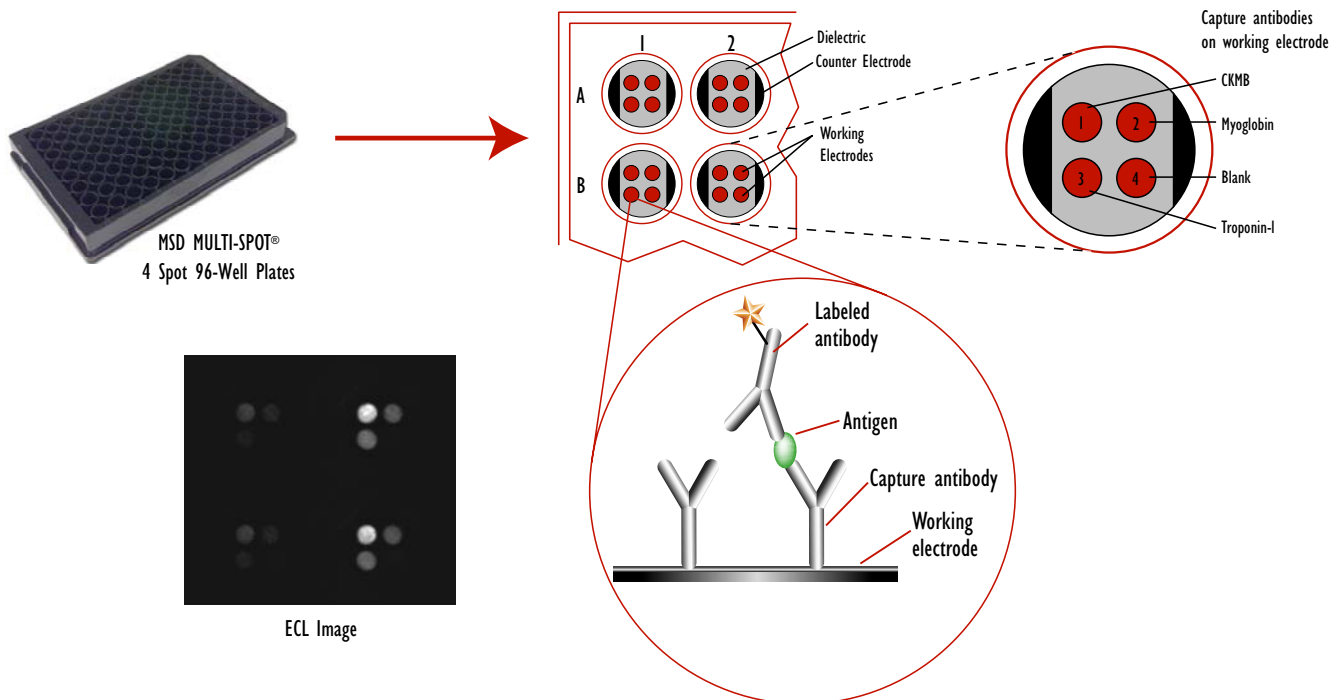
# Multiplexed Cardiac Marker Assays: CKMB, Myoglobin, and Troponin-I

## 1 Abstract

We have developed simultaneous, multiplexed assays for human cardiac Troponin-I, CKMB and Myoglobin on the MSD MULTI-ARRAY™ platform for measurement in serum. All assays are sandwich immunoassays using electrochemiluminescent-labeled detection antibodies. The assay protocol involves a single 1-hour incubation followed by a wash step. Assays are performed in MSD 96-well 4-spot plates and are read on MSD's SECTOR™ Imager 6000, at a throughput of 80 samples per minute. Assay ranges are 0.01 – 80 ng/ml (0.4 – 3000 pM) for Troponin-I, 0.15 – 400 ng/ml (1.8 – 4500 pM) for CKMB and 19 – 3000 ng/ml (1 – 170 nM) for Myoglobin. All assays are highly specific and linear over the normal clinical ranges. This multiplexed panel demonstrates the ability to perform multiplexed measurements for analytes that differ by ~100-fold in abundance.

## 2 Background and Assay Format

All three assays are formatted as sandwich immunoassays, using monoclonal antibody pairs. A 4-spot MULTI-ARRAY plate is used which is pre-coated with the three capture antibodies on separate spots within each well. Detection antibodies are labeled with a  $\text{Ru}(\text{bpy})_3^{2+}$  compound, and electrochemiluminescence is used as the detection technology.



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## 3 Assay Protocol

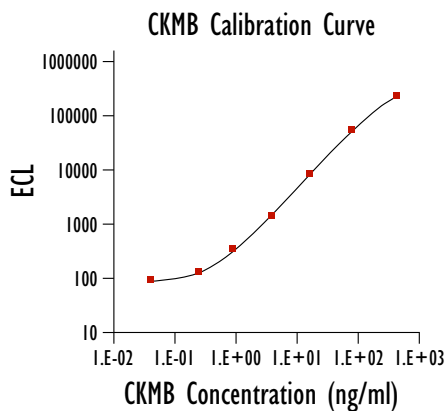
### Assay Components:

- Plate: 4-spot MULTI-ARRAY plate, pre-coated with all three capture antibodies.
- Calibration: 8-level multi-analyte calibrator set (serum based matrix).
- Detection Antibodies: labeled antibody solution containing all three labeled antibodies and additives such as HAMA blockers.

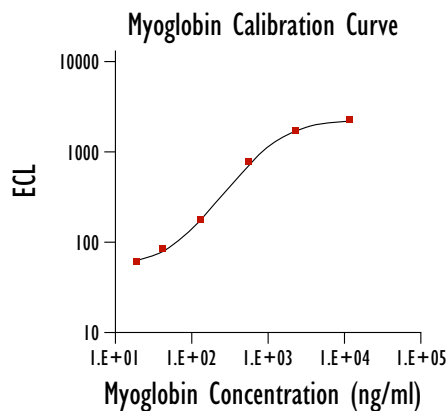
### Protocol:

- Add 25  $\mu$ l of labeled antibody solution to each well.
- Add 25  $\mu$ l of serum sample or calibrators to each well.
- Incubate with shaking for 60 minutes.
- Wash 3X with PBS.
- Add MSD-T Read Buffer.
- Read plate on SECTOR Imager 6000 Reader.

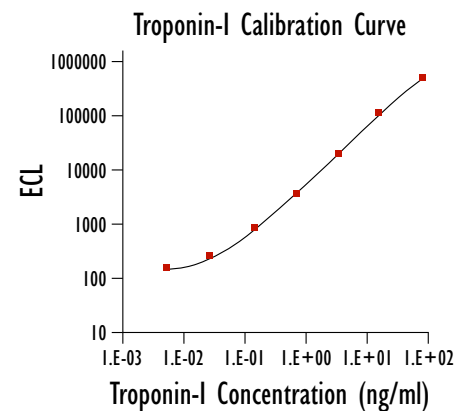
## 4 Assay Performance



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



**Sensitivity:** 19 ng/ml  
**Dynamic Range:** 19 to ~3000 ng/ml  
**Variability:** less than 10% CV



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



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## 5 Multiplexing

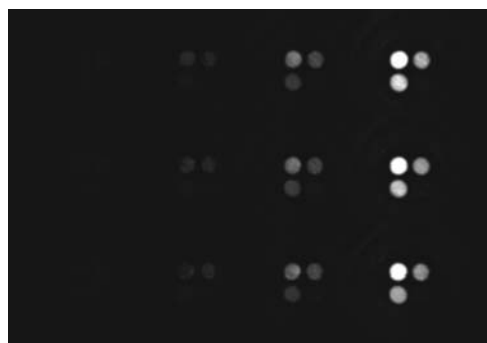
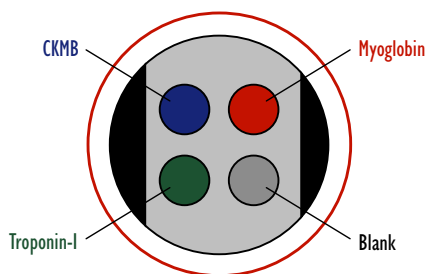
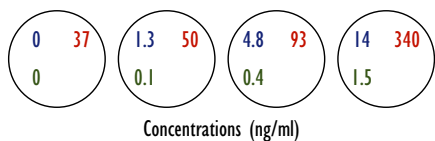


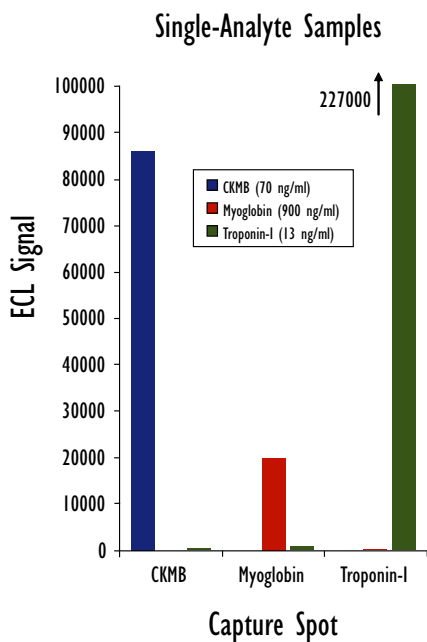
Image of ECL from multiplexed cardiac marker assays in MSD 4-Spot Plates.



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## 6 Cross-Reactivity



Inter-assay cross reactivity was evaluated by testing calibrators spiked with CKMB (70 ng/ml), Myoglobin (900 ng/ml) or Troponin-I (13 ng/ml).

Very minor cross-reactivity was noted for some assays when run in multiplex form.

	CKMB (70 ng/ml)	Myoglobin (900 ng/ml)	Troponin-I (13 ng/ml)
CKMB Spot	100%	0%	0.04%
Myoglobin Spot	0%	100%	0.2%
Troponin-I Spot	0%	0.12%	100%

## 7 Expected Values

A reference range study was performed using serum samples from healthy donors, approximately half female and half male. All twenty-four samples were measured in multiplexed format (all three analytes simultaneously) to obtain the tabulated data.

	N	Median (ng/ml)	95th Percentile	99th Percentile
CKMB	24	1.44	2.67	3.28
Myoglobin	24	27.0	47.0	57.0
Troponin-I	24	0.010	0.022	0.028



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## 8 Dilution Linearity

Serum from three individuals without history of heart disease or myocardial infarction were spiked with CKMB, Myoglobin, and Troponin-I, and then diluted with pooled human serum. Dilutions were measured three times - the average concentration for each analyte is listed in the tables.

Dilutions are linear, and average recoveries are 104% for CKMB, 104% for Myoglobin, and 104% for Troponin-I over all three samples and three dilutions.

CKMB Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	Mean Recovery Percentage
1	1/1	145.1	--	--
	1/2	72.5	73.3	101
	1/4	36.3	37.6	104
	1/8	18.1	19.6	108
2	1/1	141.8	--	--
	1/2	70.9	75.4	106
	1/4	35.4	35.3	100
	1/8	17.7	19.2	108
3	1/1	144.7	--	--
	1/2	72.3	74.7	103
	1/4	36.2	35.3	98
	1/8	18.1	19.1	105

Myoglobin Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	Mean Recovery Percentage
1	1/1	302	--	--
	1/2	161	163	101
	1/4	90.5	95.2	105
	1/8	55.2	57.4	104
2	1/1	231	--	--
	1/2	125	120	96
	1/4	72.7	76.3	105
	1/8	46.4	51.5	111
3	1/1	209	--	--
	1/2	115	109	95
	1/4	67.4	67.7	100
	1/8	43.7	50.7	116

Troponin-I Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	Mean Recovery Percentage
1	1/1	18.8	--	--
	1/2	9.4	9.9	105
	1/4	4.7	4.8	102
	1/8	2.4	2.5	107
2	1/1	21.0	--	--
	1/2	10.5	10.4	99
	1/4	5.3	5.4	103
	1/8	2.6	2.7	101
3	1/1	22.0	--	--
	1/2	11.0	11.0	100
	1/4	5.5	6.0	108
	1/8	2.8	3.1	113



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## 9 Spike Recovery

Three serum samples were spiked with known quantities of CKMB, Myoglobin, and Troponin-I and measured three times. The measured average concentration for each analyte is compared to the expected concentration in the tables.

Average recovery is 105% for CKMB, 108% for Myoglobin, and 97% for Troponin-I over all three samples and 3 spike levels.

CKMB Recovery				
Sample #	Amount Spiked (ng/ml)	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	Mean Recovery Percentage
1	0.0	1.8	--	--
	3.2	5.0	5.6	113
	9.7	11.4	12.3	108
	29.0	30.8	31.0	101
2	0.0	2.6	--	--
	3.2	5.9	6.2	106
	9.7	12.3	12.7	103
	29.0	31.6	26.8	85
3	0.0	3.8	--	--
	3.2	7.0	8.4	120
	9.7	13.5	14.8	110
	29.0	32.8	32.3	98

Myoglobin Recovery				
Sample #	Amount Spiked (ng/ml)	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	Mean Recovery Percentage
1	0	30	--	--
	33	63.3	67.5	107
	100	130	148	114
	300	330	386	117
2	0	16.3	--	--
	33	49.6	49.3	99
	100	116	120	103
	300	316	377	119
3	0	33.6	--	--
	33	66.9	66.3	99
	100	134	143	107
	300	334	370	111

Troponin-I Recovery				
Sample #	Amount Spiked (ng/ml)	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	Mean Recovery Percentage
1	0.0	0.0	--	--
	2.2	2.2	1.9	87
	6.7	6.7	5.4	81
	20.0	20.0	19.5	97
2	0.0	0.0	--	--
	2.2	2.2	2.2	99
	6.7	6.7	5.9	89
	20.0	20.0	19.3	97
3	0.0	0.0	--	--
	2.2	2.2	2.6	114
	6.7	6.7	7.0	104
	20.0	20.0	21.7	108



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## 10 Performance Summary

Analyte	MSD 4-Spot Plate Assay		Roche Elecsys (Beckman Access)	
	Analytical Sensitivity	Linear Range	Analytical Sensitivity	Linear Range
CKMB	0.15 ng/ml	400 ng/ml	0.1 ng/ml	500 ng/ml
Myoglobin	19 ng/ml	3000 ng/ml	21 ng/ml	3000 ng/ml
Troponin-I	0.01 ng/ml	80 ng/ml	(0.03 ng/ml)	(100 ng/ml)

Clinical Decision Levels:

CKMB: 5 ng/ml  
Myoglobin: 50 ng/ml  
Troponin-I: 0.1 ng/ml

Analytical sensitivity = analyte concentration at 25% of background

## 11 Conclusions

- Multiplexed CKMB, Myoglobin, and Troponin-I immunoassays have been demonstrated on the MSD platform.
- Assays are rapid (1 hour incubation), simple (one addition/one wash), use small sample volumes (25  $\mu$ l), are very sensitive and have wide dynamic ranges (>3 logs).
- Performance is comparable to clinical laboratory analyzers.



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