

# Cardiac-I Triplex: Multiplex 96-Well Plate Assays for CKMB, Myoglobin and Troponin-I



Meso Scale Discovery



Meso Scale Discovery  
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# Assay Protocol

## Assay Components:

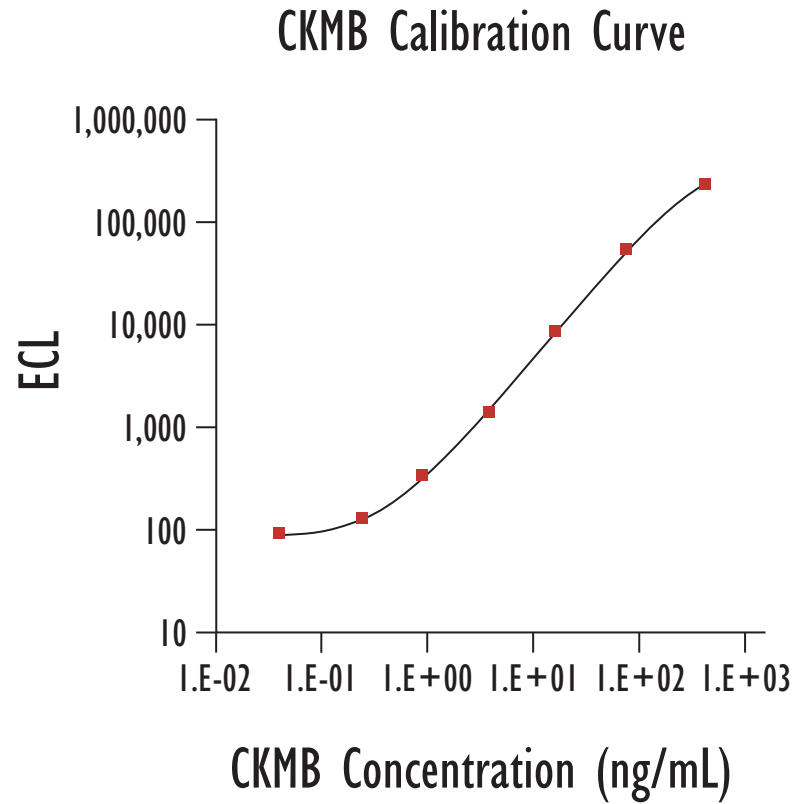
- Plate: MULTI-SPOT® 4-spot plate, pre-coated with three capture antibodies
- Calibrators: 8-level multi-analyte calibrator set (serum-based matrix)
- Detection Antibodies: labeled antibody solution containing three SULFO-TAG™ labeled antibodies and additives such as HAMA blockers

## Protocol:

- Add 25 µL of Detection Antibody Solution to each well
- Add 25 µL of serum sample or calibrators to each well
- Incubate with shaking for 60 minutes
- Wash 3X with PBS
- Add MSD Read Buffer T
- Read plate on MSD SECTOR™ Imager



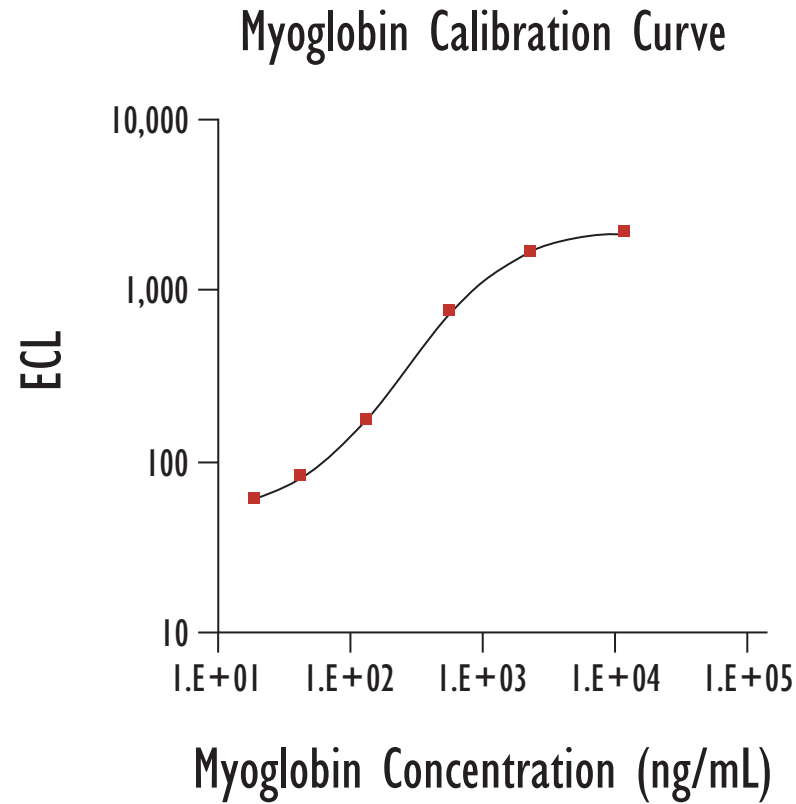
# CKMB Assay Performance



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



# Myoglobin Assay Performance

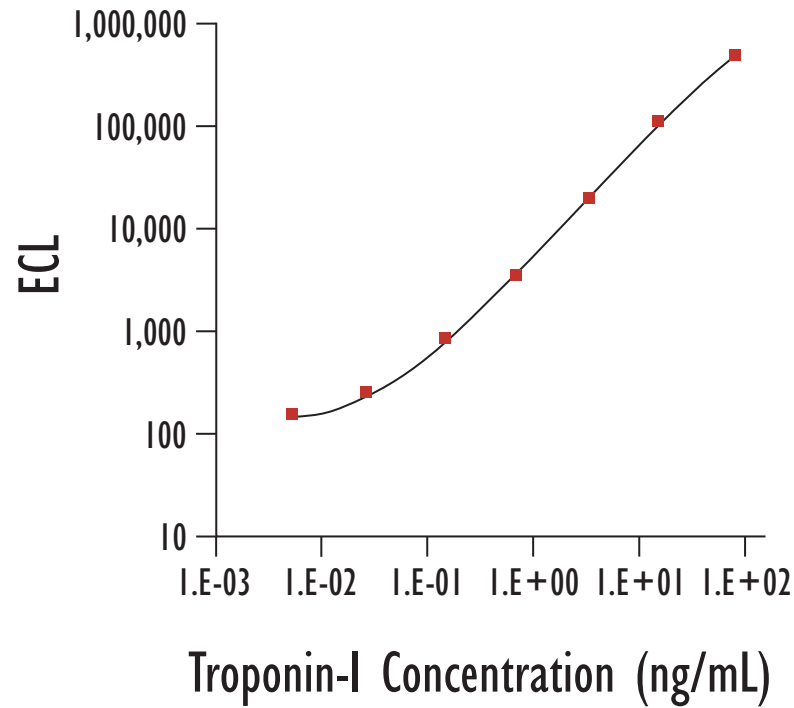


**Sensitivity:** 19 ng/mL  
**Dynamic Range:** 19 to ~3000 ng/mL  
**Variability:** less than 10% CV



# Troponin-I Assay Performance

## Troponin-I Calibration Curve



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



# 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	Mean	Median	Range
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



# 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 below.

Dilutions are linear, and the average recoveries are 114% for CKMB, 104% for Myoglobin, and 104% for Troponin-I over all three dilutions of the samples.

CKMB Dilution Linearity				
Sample #	Dilution Factor	Expected Concentration (ng/mL)	Measured Concentration (ng/mL)	Mean Recovery Percentage
1	1/1	119.4	--	--
	1/2	59.7	70.4	118%
	1/4	29.9	32.9	110%
	1/8	14.9	18.9	127%
2	1/1	118.4	--	--
	1/2	59.2	69.5	117%
	1/4	29.6	31.1	105%
	1/8	14.8	18.3	123%
3	1/1	131.2	--	--
	1/2	65.6	71.0	108%
	1/4	32.8	35.7	109%
	1/8	16.4	17.2	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%



# 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 below.

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

CKMB Recovery				
Sample #	Amount Spiked (ng/mL)	Expected Concentration (ng/mL)	Measured Concentration (ng/mL)	Mean Recovery Percentage
1	0	1.8	--	--
	3.2	5.0	5.6	113%
	9.7	11.4	12.3	108%
	29	30.8	31.0	101%
2	0	2.6	--	--
	3.2	5.9	6.2	106%
	9.7	12.3	12.7	103%
	29	31.6	26.8	85%
3	0	3.8	--	--
	3.2	7.0	8.4	120%
	9.7	13.5	14.8	110%
	29	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	--	--
	2.2	2.2	1.9	87%
	6.7	6.7	5.4	81%
	20.0	20.0	19.5	97%
2	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	--	--
	2.2	2.2	2.6	114%
	6.7	6.7	7.0	104%
	20.0	20.0	21.7	108%

# Performance Summary

Assay	MSD MULTI-SPOT Assay		Roche Elecsys (Beckmann 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	3,000 ng/mL	21 ng/mL	3,000 ng/mL
Troponin-I	0.01 ng/mL	80 ng/mL	(0.03 ng/mL)	(100 ng/mL)

Analytical sensitivity = analyte concentration at 25% of background

Clinical Decision Levels:

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



# 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 range (>3 logs).
- Performance is comparable to clinical laboratory analyzers.

