

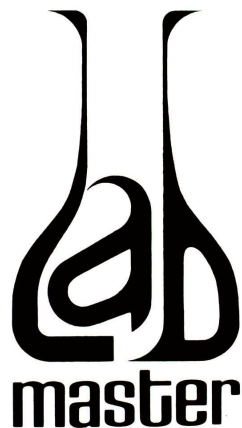
Labmaster TR-FIA tests:

1212-2001	Enterolactone kit
1212-2002	Equol kit
1212-2003	Genistein kit
1212-2004	Daidzein kit

Labmaster ELISA tests:

1212-1003	Anti-Gliadin IgG kit
1212-1004	Anti-Gliadin IgA kit
1212-1006	Troponin I kit

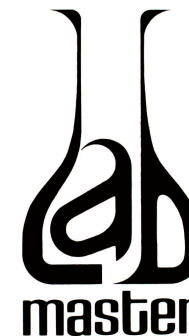
2003-03-F



LABMASTER

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This package insert must be read carefully prior to use.
Package insert instructions must be followed accordingly. Reliability of assay results can not be guaranteed if there are any deviations from the instructions in this package insert.

Labmaster Troponin I ELISA

1212-1006

INTENDED USE

This *in vitro* diagnostic method is intended to quantitatively measure the concentration of cardiac troponin I in human plasma. Troponin I values are used to assist in the diagnosis of acute myocardial infarction (AMI).

SUMMARY AND EXPLANATION OF THE TEST

Cardiac troponin I (cTnI) is a component of troponin complex, responsible for the regulation of muscle contraction.¹ The troponin complex consists of three different molecules: troponin C (TnC) with molecular weight 18.4 kDa and pI 4.1, cTnI, with molecular weight 23.9 kDa and pI 9.9, and cardiac troponin T (cTnT), with molecular weight 34 kDa and pI 5.1. All three components interact with each other with different strengths. The strongest interaction was demonstrated for cTnI-cTnT complex ($K_A = 4.4 \times 10^{-5} \text{ M}^{-1}$) and especially for cTnI-TnC complex in the presence of Ca^{2+} ($K_A = 1.5 \times 10^{-8}$)².

Cardiac troponin I is uniquely located in the myocardium and its unique amino sequence shows approximately 40% heterology from skeletal muscle isoforms.^{3,4} In patients with AMI or ischemic damage, the plasma cTnI level becomes abnormal in 4 to 6 hours, peaks in about 12 hours, and remains elevated for 3 to 10 days following AMI.⁵⁻⁷ The high specificity of cTnI measurement in the identification of cardiac injury has been shown in clinical conditions involving skeletal muscle injury resulting from surgery, trauma, extensive exercise, or muscular disease.⁸⁻¹⁰

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The assay is based on enzyme immunoassay (ELISA) technology using horseradish peroxidase as a label. Only 25 μL of plasma is needed and the hook effect is avoided by using 2-step assay format (30 min + 30 min). The substrate contains both 3,3', 5,5' tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in one bottle format with greatly enhanced stability and sensitivity. Increasing absorbance is monitored at 450 nm.

REAGENTS

MATERIALS PROVIDED

The following materials are available and provided for 48 tests in the package sizes listed in Table 1.

Table 1: Reagent packaging information

Code	Nature of the reagents	Package	Presentation
FP	Anti-TnI-IgG coated microtitration strips	1 bag	12x8 wells ready to use
	Troponin-I standards		
FSTA	0 ng/mL	1 vial	0.4 mL ready to use
FSTB	0.35 ng/mL	1 vial	0.4 mL ready to use
FSTC	0.7 ng/mL	1 vial	0.4 mL ready to use
FSTD	3.5 ng/mL	1 vial	0.4 mL ready to use
FSTE	7.0 ng/mL	1 vial	0.4 mL ready to use
FSTF	14.0 ng/mL	1 vial	0.4 mL ready to use
FAB	Assay buffer	1 vial	3 mL ready to use
SD	Sample Diluent	1 vial	40 mL ready to use
FE	Tracer Solution	1 vial	7 mL ready to use
WC	Wash Solution Concentrate	1 vial	40 mL (25 x) to be diluted in distilled water
SS	Substrate Solution	1 vial	7 mL ready to use
SPS	Stop Solution	1 vial	7 mL ready to use

1. **Anti-Troponin-I IgG Coated Strips:** 12x8 wells – Ready to use.
Microtiter wells coated with anti-human cardiac troponin-I monoclonal anti-body (mouse), stored in sealed aluminium foil bags with a desiccant bag.
2. **Troponin-I standards from 0 to 14 ng/mL:** 0.4 mL - Ready to use.
Standards are in bovine albumin solution and contain troponin-I from human heart (except standard 0 ng/m).
3. **Assay Buffer:** 3 mL - Ready to use
Contains 0.05% NaN₃
4. **Sample Diluent:** 40 mL - Ready to use
Contains 0.05% NaN₃
5. **Tracer Solution:** 7 mL - Ready to use
Contains phenol and NH₄OH, bovine proteins
6. **Wash Concentrate:** 40 mL - 25X concentrate
Contains 1 % Proclin-300
7. **Substrate Solution: 7 mL - Ready to use**
Contains 5 % N,N-Dimethyl formamide
8. **Stop Solution:** 7 mL - Ready to use
Contains 5% H₂SO₄

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use Only

SAFETY PRECAUTIONS

CAUTION: This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the REAGENTS section of this package insert. No known test method can offer complete assurance that products derived from human sources will not transmit infection. All human sourced materials should be considered potentially infectious.

Some components of this product contain Sodium Azide. For a specific listing, refer to the REAGENTS section of this package insert. Troponin-I Standards, Assay Buffer and Sample Diluent contain Sodium Azide and are classified European Community (EC) directives as: Harmful (Xn). The following are the appropriate risk (R) and safety (S) phrases.



R22	Harmful if swallowed
R32	Contact with acids liberates very toxic gas
S2	Keep out of the reach of children
S13	Keep away from food, drink and animal food
S36	Wear suitable protective clothing
S46	If swallowed, seek medical advice immediately and show this container or label

STORAGE INSTRUCTIONS

Labmaster Troponin-I ELISA Kit can be stored at +2°/+8°C until the expiry date indicated on the packaging.

1. Mouse IgG Anti-human cTnI Coated Strips:

They can be stored at +2°/+8°C until the expiry date indicated on the packing. In order to protect the strips from humidity, store them in their original bag with the desiccator.

2. Troponin I Standards:

They are specially stabilized (patent pending) and they can be stored at +2°/+8°C until the expiry date indicated on the packing. Freezing and thawing at least three times is allowed.

3. Assay Buffer, Sample Diluent, Tracer Solution, Wash Concentrate, and Stop Solution:

They can be stored at +2°/+8°C until the expiry date indicated on the labels of the vials.

4. Substrate solution:

Can be stored at +2°/+8°C until the expiry date indicated on the packaging. This solution is light sensitive; it must be stored at +2°/+8°C in the dark at all times.

5. Washing solution

After preparing the Wash Solution from Wash Concentrate, the solution can be stored for 1 month at +2°/+8°C.

MATERIALS REQUIRED – NOT SUPPLIED

- Bottle (1 L) to dilute Wash Solution Concentrate
- Precision micropipettes to deliver 25 µL
- Distilled water
- Precision 8-channel multipipette or dispenser to deliver 25 µL and 50 µL
- Microwell shaker
- Automatic platewasher
- ELISA plate reader with 450 nm filter
- Test tubes for possible sample dilutions

TROPONIN-I ELISA PROCEDURE

Perform each determination in duplicate for both standards and unknowns. A standard curve should be run with each assay. All reagents must be brought to room temperature (+20- +25°C) before use.

1. Preparation of Wash solution

Pour the 40 mL of Wash Concentrate into a clean container and dilute 25-fold by adding 960 mL distilled water to give wash solution

2. Transfer the required number of microtitration strips to a strip frame. (Return the remaining strips to the plastic tray pack and reseal.)

3. Pipette 25 µL of Assay Buffer into the strip wells.

- Pipette 25 μL of the Troponin-I Standards (Std) and patient specimens (unknowns – Unk) into the strip wells according to the following table.

	1	2
A	Std 0	Std 7.0
B	Std 0	Std 7.0
C	Std 0.35	Std 14.0
D	Std 0.35	Std 14.0
E	Std 0.7	1st Unk
F	Std 0.7	1st Unk
G	Std 3.5	etc.
H	Std 3.5	

- Incubate the frame for 30 min at room temperature with slow shaking.
- Aspirate and wash each strip 6 times with a washing device.
- Add 50 μL of Tracer Solution to each well.
- Incubate the frame for 30 min at room temperature with slow shaking.
- Aspirate and wash 6 times each strip with a washing device.
- Add 50 μL of Substrate Solution and incubate the frame for 15 min at room temperature with slow shaking.
- Add 50 μL of Stop Solution shake the frame for 5 s and measure the absorbance in the microplate reader at 450 nm within 10 minutes.

PROCEDURAL NOTES

- A thorough understanding of this package insert is necessary for successful use of the ELISA kit. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use kit reagents after the expiry date printed on the kit label.
- Reagents should be allowed to reach room temperature (+20 - +25°C) prior to sample preparation. Frozen patient specimens should be brought to room temperature slowly and gently mixed by hand. Do not vortex or vigorously mix patient specimens.
- When washing the strips ensure that each well is filled up completely to the top edges as shown in the figure. After washing the strips, check that the wells are dry. If there is moisture left, invert the plate and tap firmly against absorbent paper.
- The aspirate/wash device should not left standing with wash solution for long periods, as the needles may get clotted giving poor liquid delivery and suction. At the end of the working day aspirate distilled water into the aspirate/wash device. The washing device should be regularly rinsed with NaCl solution (30g/L), followed by distilled water and 50% ethanol and then finally flushed with distilled water.

CALCULATION OF RESULTS

Calculate the mean of the duplicate absorbance determinations. Construct a standard curve on graph paper by plotting the mean absorbances of the six standards (ordinate) against the corresponding cTnI concentration (abscissa). Draw the best fitting curve.

Programs for data reduction are recommended in order to obtain the results as printouts of standard curves, unknown concentrations etc.

Determine cTnI concentration of each of the patient specimens and controls by interpolation on the curve.

Patient specimens giving absorbance values below the highest standard (14.0 ng/mL) should be pre-diluted 1:20 (1+19) with Sample Diluent, and then re-tested according to the assay procedure. After assaying, multiply the result by the dilution factor.

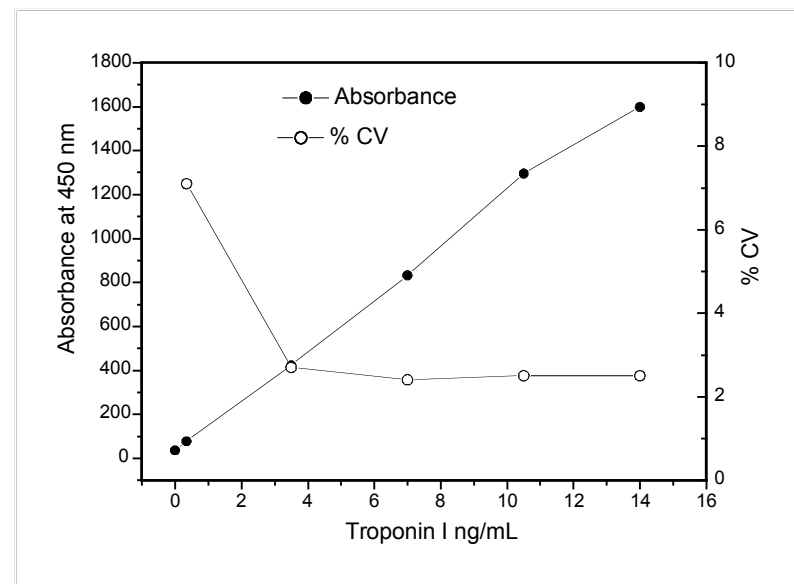
LIMITATIONS OF THE PROCEDURE

For diagnostic purposes the troponin-I ELISA results should be used in conjunction with other data; e.g. other clinical testing, ECG, symptoms, clinical observations, etc.

So-called "Spontaneous HAMAs"-samples that are found in people revealing high HAMA titers without any reasonable explanation should not be assayed with Labmaster Troponin I ELISA kit.

SPECIFIC PERFORMANCE CHARACTERISTICS

A typical standard curve and precision profile for troponin-I ELISA kit are shown below.



Inter-assay (between run) precision of the troponin-I ELISA assay is shown below:

Sample	Number of duplicate determinations	Mean value ng/mL	% CV
1	21	0.73	19.5
2	21	3.45	6.4
3	21	7.07	3.7

RECOVERY

Human serum containing a known concentration of troponin-I was used to supplement three normal human serum specimens (with undetectable troponin-I values) at three concentration levels. The concentration of troponin-I was determined using Troponin-I ELISA Kit and the resulting percent recovery was calculated.

Specimen	Troponin-I Value Expected (ng/mL)	Troponin-I Value Obtained (ng/mL)	% Recovery ^a
1	14.4	13.2	91.7
	7.2	6.4	88.9
	3.6	3.7	102.8
2	14.4	13.6	94.4
	7.2	7.4	102.8
	3.6	3.5	97.2
3	14.4	13.1	91.0
	7.2	7.1	98.6
	3.6	3.9	108.3

Average % Recovery 97.3%

$${}^a \text{ \% Recovery} = \frac{(\text{Troponin-I Value Obtained})}{(\text{Troponin-I Value Expected})} \times 100$$

DILUTION LINEARITY

Dilution linearity was investigated by serial dilution of three samples of known troponin-I concentrations. All samples were diluted with Sample Diluent of Troponin-I ELISA Kit.

Sample	Final Dilution Factor	Observed (ng/mL)	Method 1 %Recovery ^a	Method 1 %Recovery ^b
1	8	18.7		
	16	9.9	105.9	105.9
	32	4.8	97.0	102.7
	64	2.2	91.7	94.1
2	128	1.1	100.0	94.1
	8	18.5		
	16	8.6	93.0	93.0
	32	4.8	111.6	103.8
3	64	2.1	87.5	90.8
	128	1.0	95.2	86.5
	4	18.8		
	8	10.1	107.4	107.4
4	16	4.9	97.0	104.3
	32	2.4	98.0	102.1
	64	1.3	108.3	110.6
	2	14.2		
5	4	6.8	95.8	95.8
	8	4.0	117.6	112.7
	16	2.0	100.0	112.7
	32	1.0	100.0	112.7

$$^a\text{Method1: \%Recovery} = \frac{\text{Observed Value}}{(\text{Previously Observed Value}/2)} \times 100$$

$$^b\text{Method2: \%Recovery} = \frac{\text{Observed Value}}{(\text{First Value} \times \text{Dilution Factor}/\text{Dilution Factor})} \times 100$$

CROSS REACTIVITY

No cross-reaction has been found with skeletal muscle troponin I

ANALYTICAL SENSITIVITY

The analytical sensitivity of Troponin-I ELISA Kit was calculated to be 0.171 ng/mL at the 95% level of confidence (n = 32). Analytical sensitivity was determined as the concentration at two standard deviations from the mean of 32 replicates of the standards of Troponin-I ELISA Kit and represents the lowest measurable concentration of cardiac troponin-I can be distinguished from zero.

CORRELATION DATA

The troponin I ELISA assay was compared to a commercially available diagnostic kit. The results of the linear regression analysis are shown below.

Method	Number of Observations	Intercept	Slope	Correlation coefficient
Labmaster Troponin I kit vs. Bayer Immuno 1 System Troponin I	122	-1.911	1.10	0.95

REFERENCES

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