ab326433 – Human Cardiac Troponin I SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of Cardiac Troponin I in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture media.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326433

Storage and Stability: Store kit at 2-8ºC immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity (plus any sample up-validation, native linearity, or individual donors testing results if available) were collected using the colorimetric version of this kit (ab200016).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human Cardiac Troponin I Capture Antibody 10X	600 µL	+4ºC
Human Cardiac Troponin I Detector Antibody 10X	600 µL	+4ºC
Human Cardiac Troponin I Lyophilized Recombinant Protein	2 Vials	+4ºC
Antibody Diluent CPI2	6 mL	+4ºC
Sample Diluent 50BP	20 mL	+4ºC
Sample Diluent NS	50 mL	+4ºC
Wash Buffer PT 10X	20 mL	+4ºC
ChemiHRP Reagent A	3 mL	+4ºC
ChemiHRP Reagent B	3 mL	+4ºC
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4ºC
Plate Seal	1	+4ºC

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25ºC) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the **Cardiac Troponin I** protein standard by adding the volume indicated on the protein vial label. For **cell culture media samples measurements**, use Sample Diluent NS. For **serum and plasma samples measurements**, use Sample Diluent 50BP. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 24,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 125 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	125	125	24000	12000
2	Standard#1	75	150	12000	4000
3	Standard#2	75	150	4000	1333.3
4	Standard#3	75	150	1333.3	444.44
5	Standard#4	75	150	444.44	148.15
6	Standard#5	75	150	148.15	49.38
7	Standard#6	75	150	49.38	16.46
8	Standard#7	75	150	16.46	5.48
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤100%
Plasma – Citrate*	≤100%
Plasma – EDTA*	≤100%
Plasma – Heparin*	≤100%
Cell Culture Media*	≤25%

*Based on spiked sample.

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Assay, or dilute samples into 50BP and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute samples into 50BP and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4ºC.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Cardiac Troponin I

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant.

100% pooled serum and plasma (citrate, EDTA, Heparin) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

Urine, saliva, milk, and CSF samples have not been tested with this kit.

This kit is incompatible with cell extract, and tissue extract samples.

For the measurement of Cardiac Troponin I in cell extract, and tissue extract sample types, use Human Cardiac Troponin I ELISA kit ab200016.

CROSS REACTIVITY

Recombinant human Cardiac Troponin C was prepared at 1 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human Cardiac Troponin C was prepared at multiple concentrations and tested for interference with 1 ng/mL recombinant human Cardiac Troponin 1.

Cardiac Troponin C	% Interference
≥2 ng/mL	10
≤1 ng/mL	<1

SPECIES REACTIVITY

This kit recognizes human Cardiac Troponin 1 protein.

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.00	132	132	132
5.49	1,010	753	882
16.46	2,360	2,600	2,480
49.38	9,062	9,064	9,063
148.15	30,880	30,583	30,732
444.44	94,235	100,216	97,226
1,333.3	298,257	333,479	315,868
4,000	983,041	981,778	982,410
12,000	3,002,305	3,120,967	3,061,636

Table 1. Example of human Cardiac Troponin I standard curve in 50BP. The Cardiac Troponin I standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.00	239	239	239
5.49	2,183	1,922	2,053
16.46	4,800	5,056	4,928
49.38	16,632	17,617	17,125
148.15	46,578	51,614	49,096
444.44	170,149	167,322	168,736
1,333.3	533,273	611,933	572,603
4,000	1,542,697	1,545,816	1,544,257
12,000	4,179,972	4,115,871	4,147,922

Table 2. Example of human Cardiac Troponin I standard curve in NS. The Cardiac Troponin I standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	12	0.78 pg/mL
Sample Diluent 50BP	12	0.87 pg/mL

Recovery

Three concentrations of Cardiac Troponin I were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
100% Serum	97	93 – 101
100% Plasma – Citrate	93	87 – 100
100% Plasma – Heparin	107	99 – 117
100% Plasma – EDTA	93	90 – 97
25% Cell Culture Media*	102	97 – 108

*Media is RPMI-1640 containing 10% fetal bovine serum

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant Cardiac Troponin 1 was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent 50BP for serum and plasma samples and Sample Diluent NS for cell culture media samples.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (EDTA)	100% Human Plasma (Heparin)	25% Media*
Undiluted	pg/mL	1,621	1,629	1,559	1,439	1,510
	% Expected value	100	100	100	100	100
2	pg/mL	834.5	851.5	755.6	852.0	673.0
	% Expected value	103	105	97	118	89
4	pg/mL	411.2	425.1	397.7	426.2	326.6
	% Expected value	101	104	102	118	87
8	pg/mL	210.3	216.1	229.1	214.7	159.2
	% Expected value	104	106	118	119	84
16	pg/mL	98.42	111.8	111.2	NL	77.08
	% Expected value	97	110	114	NL	82

NL – Non-Linear

*Media is RPMI1640 containing 10% fetal bovine serum.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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