ImmunoDiagnostics Limited

Mouse/rat C-peptide immunoassay kit

Catalogue number: 36780

For the quantitative determination of C-peptide
in mouse/rat serum and plasma

This package insert must be read in its entirety before using this product

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FOR RESEARCH USE ONLY

Version: 2.0

TABLE OF CONTENT

PRINCIPLE OF THE ASSAY

This assay is a two-site ELISA. The micro-plate is pre-coated with a monoclonal antibody against C-peptide. Standards and samples are added into the wells and co-incubated with a biotin labeled monoclonal antibody. After wash step to remove any unbound reagents, streptavidin-HRP conjugate (STP-HRP) is added. After the last wash step, TMB substrate is added and colour develops in proportion to the amount of C-peptide bound initially. The assay is stopped and the optical density of the wells determined using a micro-plate reader. Since the increases in absorbance are directly proportional to the amount of captured C-peptide, the unknown sample concentration can be interpolated from a reference curve included in each assay.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a monoclonal antibody against C-peptide, sealed.
2. (10×) Wash buffer-40 ml.
3. Assay buffer-25 ml, ready for use.
4. (100×) Detection antibody solution-A biotin labeled monoclonal antibody against C-peptide (0.12 ml).
5. C-peptide standard solutions- 0 ng/ml (1 ml). 0.3 ng/ml, 0.6 ng/ml, 1.2 ng/ml, 2.4 ng/ml and 6 ng/ml (0.15 ml each), ready for use.
6. 200×STP-HRP solution- 0.06 ml.
7. Substrate solution- 12 ml, ready for use.
8. Stop solution-12 ml, ready for use.
9. Plate cover-2.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Horizontal micro-plate shaker capable of 600 rpm.

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the micro-plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

B. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

C. 1×STP-HRP solution.

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with Assay buffer, 100 µl of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

SAMLE HANDLING

If a sample has a C-peptide level greater than the highest standard, the sample should be diluted with 0 ng/ml C-peptide standard solution and the assay should be repeated.

ASSAY PROCEDURE

It is recommended that all standards and samples should be run in duplicate.

1. Add 100 µl of 1x Detection antibody solution per well.
2. Add 10 µl of standard or sample to its respective well.
3. Seal the plate with a plate cover. Incubate at room temperature for 60 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
4. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1× Wash buffer to each well. Incubate at room temperature for 1 minute. Discard the 1× Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
5. Add 100 µl of 1×STP-HRP solution to each well, seal the plate with a plate cover. Incubate at room temperature for 30 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
6. Wash each well 4 times as described in step 2.
7. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**.
8. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

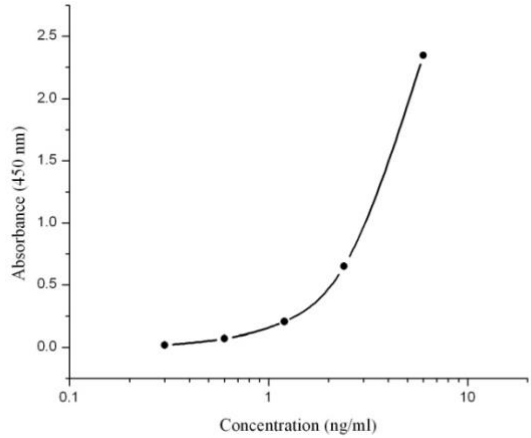
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against C-peptide concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Log-log curve fitting or curve of 4-parameter can be used for calculation.
3. Determine C-peptide concentration of samples from standard curve.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

C-peptide (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.133	0
0.3	0.151	0.018
0.6	0.203	0.07
1.2	0.338	0.205
2.4	0.783	0.65
6	2.479	2.346

C-peptide standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest C-peptide level that can be measured by this assay is 0.3 ng/ml.

B. Precision
Intra-assay Precision (Precision within an assay) C.V <4.2%.
Inter-assay Precision (Precision between assays) C.V <7.5%.

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of 1× Detection antibody solution per well.
↓
Add 10 µl of standard or sample to each well.
↓
Incubate at room temperature for 60 minutes (600 rpm).
↓
Wash each well 3 times.
↓
Add 100 µl of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 30 minutes (600 rpm).
↓
Wash each well 4 times.
↓
Add 100 µl of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µl of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation

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