

PRODUCT INFORMATION & MANUAL

Human MCP-1 Instant ELISA

BMS281INST

Enzyme-linked immunosorbent assay for
quantitative detection of human MCP-1.

For research use only.

Not for diagnostic or therapeutic procedures.

128 Tests



*Human MCP-1
Instant ELISA*

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TABLE OF CONTENTS

1	Intended Use	3
2	Summary	3
3	Principles of the Test	4
4	Reagents Provided	5
5	Storage Instructions	6
6	Specimen Collection	6
7	Materials Required But Not Provided	7
8	Precautions for Use	8
9	Preparation of Reagents and Samples	10
10	Test Protocol	11
11	Calculation of Results	15
12	Limitations	19
13	Performance Characteristics	20
14	Ordering Information	24
15	Reagent Preparation Summary	25
16	Test Protocol Summary	27

1 Intended Use

The human MCP-1 Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human MCP-1. **The human MCP-1 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

Chemokines (chemoattractant cytokines) represent a superfamily of small secreted proteins that function as intercellular messengers to control migration and activation of leukocytes involved in inflammatory reactions and immunity. Furthermore, chemokines are important mediators of many pathologies such as allergic responses, chronic inflammatory and autoimmune diseases, tumor growth and hematopoietic development.

The CC-chemokine monocyte chemoattractant protein 1 (MCP-1), also known as monocyte chemoattractant and activating factor (MCAF) was characterized as a monocyte-specific chemoattractant that was later shown to attract also T lymphocytes and NK cells.

The human mature MCP-1 protein consists of 76 amino acids, derived by cleavage of a hydrophobic signal peptide from the 99 aa precursor protein. MCP-1 is mainly expressed by macrophages in response to a wide range of cytokines such as IL-6, TNF- α and IL-1 β , but can, upon stimulation, also be produced by a variety of cells and tissues, such as fibroblasts, endothelial cells or certain tumor cells.

Because of its target cell specificity, MCP-1 was postulated to play a pathogenic role in a variety of diseases characterized by mononuclear cell infiltration, including atherosclerosis, rheumatoid arthritis and allergic responses. Elevated levels of MCP-1 have also been found in connection with osseous inflammation and Alzheimer's disease (AD) as well as Myocardial Ischemia and viral infections.

In acute and chronic-active multiple sclerosis (MS) lesions immunoreactivity for MCP-1 was increased whereas MCP-1 was found to be significantly reduced in cerebrospinal fluids (CSF) and chronic lesions of patients with MS. In basophils, MCP-1 is highly effective as a stimulus of histamine release but has only weak chemotactic activity. Additionally it has been shown to chemoattract CD4+ and CD8+ T lymphocytes, and expression of the MCP-1 chemokine may affect HIV infection via signaling through the CCR2 receptor.

For literature update refer to www.eBioscience.com

3 Principles of the Test

An anti-human MCP-1 coating antibody is adsorbed onto microwells. Human MCP-1 present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated anti-human MCP-1 antibody binds to human MCP-1 captured by the first antibody.

Following incubation unbound enzyme conjugated anti-human MCP-1 is removed during a wash step and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of soluble human MCP-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human MCP-1 standard dilutions and human MCP-1 sample concentration determined.

Figure 1

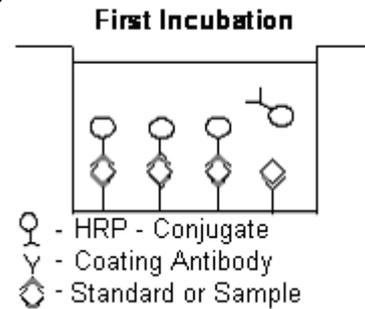


Figure 2

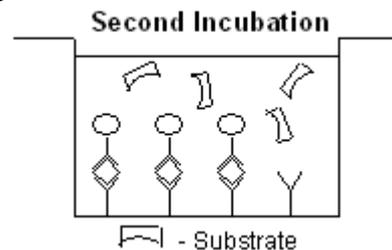
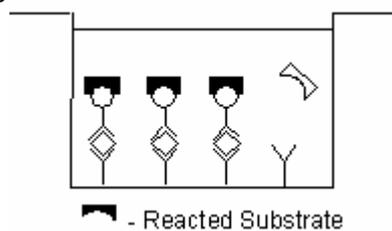


Figure 3



4 Reagents Provided

- 1 aluminium pouch with a **Microwell Plate coated** with monoclonal antibody to human MCP-1, **HRP-Conjugate** (human MCP-1 monoclonal antibody) and Assay Buffer, lyophilized
- 2 aluminium pouches with a human MCP-1 **Standard curve** (coloured)
- 1 bottle (25 ml) **Wash Buffer Concentrate 20x** (phosphate-buffered saline with 1% Tween 20)
- 1 vial (5 ml) **Assay Buffer Concentrate 20x** (Use when an external predilution of the samples is needed)
- 1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
- 2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant, serum, plasma (EDTA, heparin) and amniotic fluid were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive human MCP-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 μ l to 1000 μ l adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 μ l and 500 μ l) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

Buffer concentrates should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in buffer concentrates, warm them gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer is stable for 30 days.

9.2 Assay Buffer (1x)

Pour the entire contents (5ml) of the Assay Buffer Concentrate (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently.

10 Test Protocol

- Use plate immediately after removal from -20°C!
 - Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!
 - Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.
 - Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.
 - Allow the washing buffer to sit in the wells for a few seconds before aspiration.
 - Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.
- a. Determine the number of microwell strips required to test the desired number of samples plus microwell strips for blanks and standards (coloured). Each sample, standard and blank sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).
 - b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).
 - c. Add 130 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (1000.0 pg/ml)	Standard 1 (1000.0 pg/ml)	Sample 1	Sample 1
B	Standard 2 (500.0 pg/ml)	Standard 2 (500.0 pg/ml)	Sample 2	Sample 2
C	Standard 3 (250.0 pg/ml)	Standard 3 (250.0 pg/ml)	Sample 3	Sample 3
D	Standard 4 (125.0 pg/ml)	Standard 4 (125.0 pg/ml)	Sample 4	Sample 4
E	Standard 5 (62.5 pg/ml)	Standard 5 (62.5 pg/ml)	Sample 5	Sample 5
F	Standard 6 (31.3 pg/ml)	Standard 6 (31.3 pg/ml)	Sample 6	Sample 6
G	Standard 7 (15.6 pg/ml)	Standard 7 (15.6 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- d. Add 20 µl of each **sample**, in duplicate, to the **designated wells** and mix the contents.
- e. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours, if available on a microplate shaker at 400 rpm.
- f. Remove adhesive film and empty wells. **Wash** the microwell strips 3 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

- g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
- h. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

- i. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

- j. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human MCP-1 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human MCP-1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human MCP-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human MCP-1 concentration.
- ***Samples have been diluted 1:5, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 5).**
- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human MCP-1 levels. Such samples require further external predilution according to expected human MCP-1 values with Assay Buffer (1x) in order to precisely quantitate the actual human MCP-1 level.**
- It is suggested that each testing facility establishes a control sample of known human MCP-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 μl to the final volume per well. These 100 μl are composed of 80 μl of Assay Buffer (1x) plus 20 μl of the sample. This is a 1:5 dilution.

The remaining 50 μl to give 150 μl are due to the addition of 50 μl conjugate to all wells.

80 μl Assay Buffer (1x) and 50 μl conjugate results in 130 μl reconstitution volume, addition of 20 μl sample (80 μl + 20 μl sample= 1:5 dilution)

Figure 4

Representative standard curve for human MCP-1 Instant ELISA. Human MCP-1 was diluted in serial 2-fold steps in Assay Buffer (1x). Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

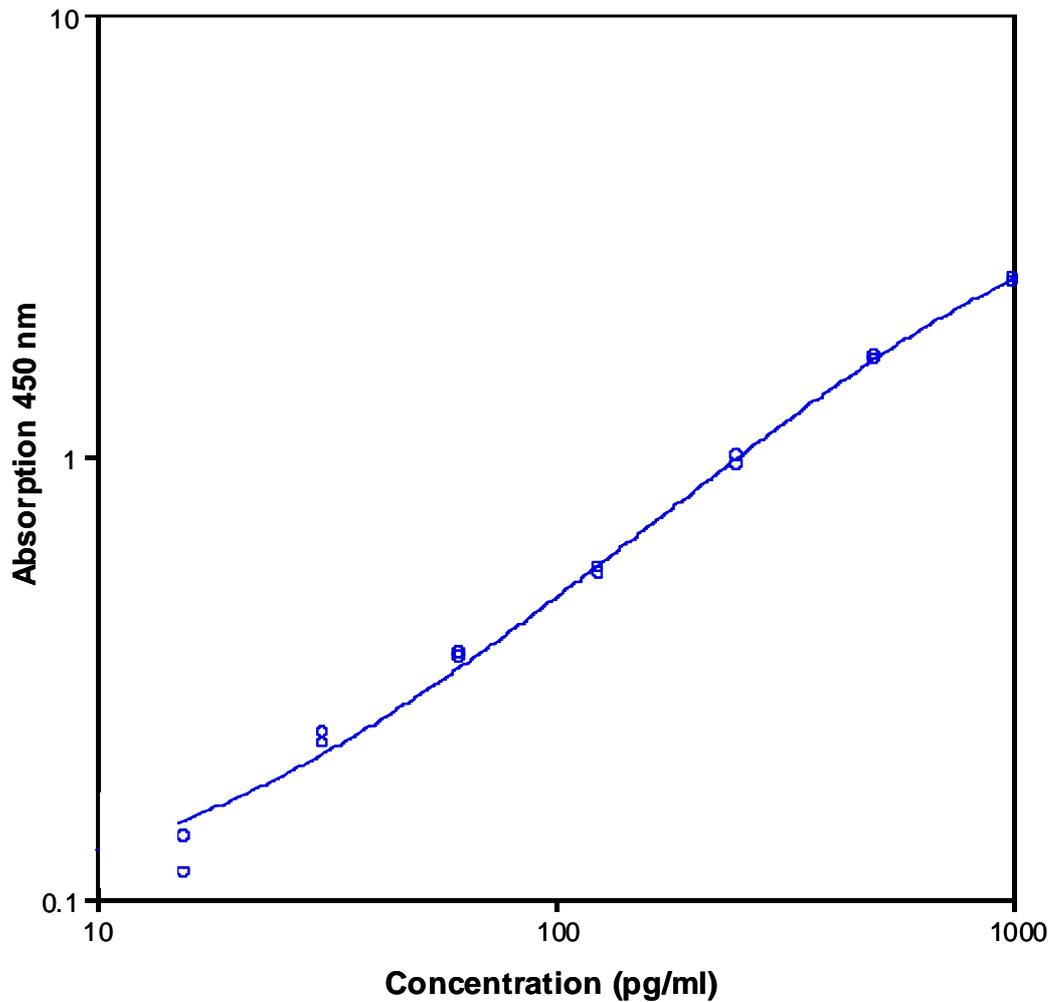


Table 2

Typical data using the human MCP-1 INSTANT ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human MCP-1 Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	1000.0	2.489 2.526	2.508	1.7
2	500.0	1.650 1.687	1.669	4.8
3	250.0	0.958 1.005	0.982	4.2
4	125.0	0.561 0.546	0.554	3.9
5	62.5	0.351 0.360	0.356	5.0
6	31.3	0.225 0.238	0.232	6.9
7	15.6	0.138 0.115	0.127	9.2
Blank	0.0	0.060 0.049	0.055	10.1

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human MCP-1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 2.31 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human MCP-1. 2 standard curves were run on each plate. Data below show the mean human MCP-1 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 7.7%.

Table 3

The mean human MCP-1 concentration and the coefficient of variation for each sample.

Sample	Experiment	Human MCP-1 Concentration (pg/ml)	Coefficient of Variation (%)
1	1	2643	10
	2	2348	11
	3	2300	7
2	1	1094	9
	2	1033	9
	3	966	10
3	1	531	9
	2	491	9
	3	448	10
4	1	181	8
	2	182	9
	3	186	7
5	1	112	9
	2	113	3
	3	104	8
6	1	83	8
	2	81	1
	3	71	2

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human MCP-1. 2 standard curves were run on each plate. Data below show the mean human MCP-1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 6.2%.

Table 4

The mean human MCP-1 concentration and the coefficient of variation of each sample

Sample	Mean human MCP-1 Concentration (pg/ml)	Coefficient of Variation (%)
1	2430	7.6
2	1033	6.5
3	490	8.5
4	183	1.4
5	110	4.7
6	78	8.2

13.3 Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human MCP-1 into serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments.

Average recovery ranged from 87% to 121% with an overall mean recovery of 104.

13.4 Dilution Parallelism

Serum samples with different levels of human MCP-1 were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged between 83% and 96% with an overall recovery of 90%.

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human MCP-1 levels determined.

There was no significant loss of human MCP-1 immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human MCP-1 level determined after 24 h.

There was no significant loss of human MCP-1 immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human MCP-1.

The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human MCP-1 positive sample.

There was no cross reactivity or interference detected.

13.7 Expected Values

A panel of randomly selected sera from blood donors was tested for human MCP-1. The detected human MCP-1 levels ranged between 74 and 760 pg/ml.

14 Ordering Information

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15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20 x (25 ml) to 475 ml distilled water

15.2 Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20 x (5 ml) to 95 ml distilled water

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 130 μ l distilled water to sample wells.
- Add 20 μ l sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) if available on a microplate shaker at 400 rpm.
- Empty and wash microwell strips 3 times with 400 μ l Wash Buffer.
- Add 100 μ l of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 100 μ l Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Samples have been diluted 1:5, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 5)