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ELISA for quantification of macrophage-colony stimulating factor (M-CSF) in human serum or plasma.

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1 An anti-human macrophage-colony stimulating factor (M-CSF) coating antibody is adsorbed onto

the microwells by incubation overnight at 4°C with carbonate-bicarbonate buffer.

- 2** Add 50 µl of human serum or plasma. M-CSF present in the serum sample binds to antibodies adsorbed into the microwells.
- 3** The microplate is blocked with 3% non-fat milk-PBS buffer and later wash to remove unbound proteins.
- 4** Fifty (50) µl of biotin-conjugated anti-M-CSF antibody is added. The optimal dilution must be investigated.
- 5** The microplate is rewashed with PBS-Tween 20 buffer, pH 7.4.
- 6** One hundred µl of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-M-CSF antibody.
- 7** The plate is washed following incubation to remove the unbound Streptavidin-HRP conjugate.
- 8** Add 100 µl of 3,3',5,5'- tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.
- 9** Incubate the microwells in the dark for 15 min.

- 10** A colored product is formed in proportion to the quantity of M-CSF present in the sample or standard.
- 11** The reaction is terminated by addition of 100 μ l 3M H₂SO₄ and the absorbance is measured at 450 nm.
- 12** A standard curve is made from 7 human M-CSF standard dilutions and the human M-CSF sample concentration is determined.
- 13** For better results place the microplate on a microplate shaker in every incubation.