Version 11-09/25

User's Manual

Romiplostim ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Romiplostim (Nplate™) ELISA is a rapid and easy method for the quantitative determination of romiplostim in human serum, plasma and cell culture supernatant.

This instruction manual is for example only. Please refer to the instruction manual upon delivery.

General Description

Romiplostim (Nplate™) for immune thrombocytopenia (ITP) and acute radiation syndrome (ARS) Romiplostim, marketed as Nplate™, is a thrombopoietin receptor agonist (TPO-RA) approved for the treatment of:

Immune Thrombocytopenia (ITP): Nplate™ is used in adult patients with ITP who have not responded sufficiently to other treatments like corticosteroids, immunoglobulins, or splenectomy. It's also approved for pediatric patients aged 1 year and older who have had ITP for at least 6 months and haven't responded to these treatments.

Acute Radiation Syndrome (ARS): Nplate™ is indicated to increase the survival of adults and children exposed to high levels of radiation (specifically, the hematopoietic syndrome of acute radiation syndrome).

Principles of Testing

An anti-Romiplostim coating antibody is adsorbed onto a microtiter plate. Romiplostim present in the sample or standard binds to the antibodies adsorbed on the plate, a biotinylated anti-Romiplostim antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin enzyme conjugate is added and binds to the biotinylated anti-Romiplostim antibody. Unbound streptavidin enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of Romiplostim 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 Romiplostim and sample concentration is then determined.

Reagents And Materials Provided

1. Anti-Romiplostim Coated Plate: One strip well 96-well plate.
2. Anti-Romiplostim Biotin
3. Streptavidin-enzyme Conjugate
4. Romiplostim Standard
6. Wash Buffer
7. Substrate Solution
8. Stop Solution

Materials Required But Not Supplied

1. Sample: plasma, serum.
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips.
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips.
4. Multichannel micropipette reservoir.
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length).
6. Ultrapure wate

Storage

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label.

Assay Procedure

1. Before performing the assay, samples and assay kit should be brought to room temperature (about 30 minutes beforehand) and ensure the homogeneity of the solution.
2. Add 100 µL of sample or standard to the Anti-Romiplostim Antibody Coated Plate. Each sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 300 µL 1× Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1× Wash Buffer.
5. Add 100 µL of the diluted Anti-Romiplostim Biotin to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. incubate at room temperature for 15 minutes.
12. Stop the enzyme reaction by adding 50 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Sensitivity

15.6-1000pg/ml