Quick Guide – quantification of TNF-alpha inhibitor biological activity

1 Plate 1

- Equilibrate reagents and samples to room temperature – **do not thaw cells and substrate reagents at this stage**
- Dilute samples according to Table 2.
- 100 µL calibrators, controls and samples are added to plate 1 (polypropylene). One well per sample at this step, to be split to duplicates in step 3 (plate 2)
- 100 µL of TNF-alpha is added to all wells in use (calibrators, controls and samples)
- Cover the wells and mix the contents

2 Incubation 1 30 min

- Incubate at 37 °C with 5% CO_2 for 30 min.
- 5 minutes before the end of the incubation, thaw and dilute the cells; add entire cell contents to 6 mL Diluent B
- Commence thawing of substrate reagents

3 Plate 2

- 50 µL sample/TNF alpha solutions are transferred to plate 2 (white plate) in duplicate
- 50 µL diluted cells are added to all wells in plate 2
- Cover the wells and mix the contents

4 Incubation 2 3 h

- Incubate at 37 °C with 5% CO_2 for 3 hours.

5 Read plate 2

- Equilibrate plate 2 to room temperature
- Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix. Protect the plate from light. Read in a luminometer after 10 min incubation.
- If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix. Protect the plate from light. Read in a luminometer after 10 min incubation.

Troubleshooting, FAQ and Contacts

Please consult Euro Diagnostica's website www.eurodiagnostica.com

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