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Sample Preparation for the Measurement of TGF-β for use on the Luminex System

Overview

The inactive form of TGF-beta is a homodimer that is non-covalently linked to a latency-associated peptide homodimer (LAP). The active form is a homodimer of mature TGF-β1 that is disulfide linked. TGF-beta, in vivo, is processed from a latent form to the bioactive form of the protein. Only the bioactive form of TGF-beta is immunoreactive and detected in our assay.

This procedure described here is used for preparing samples to be quantitatively measured for TGF-β on the Luminex platform.

Required Reagents

- 1N HCL (100mL)- To 91.67 mL of deionized water, slowly add 8.33 mL of 12N HCL.
- 1.2N NaOH/0.5M HEPES (100 mL)-To 75 mL of deionized water, slowly add 12 mL of 10N NaOH.
- Mix well. Add 11.9 g of HEPES.
- Mix well. Bring final volume to 100 mL with deionized water.

For each new lot of acidification and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that it is within pH 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed.

TGF-β Sample Activation Procedure

Note: Do not activate kit standards.

Cell Culture Supernatants

1. To 100 µL of cell culture supernatants, add 20 µL of 1N HCL
2. Mix well
3. Incubate 10 minutes at RT.
4. Neutralize acidified sample by adding 13 µL of 1.2N NaOH/0.5M HEPES
5. Mix well
6. Proceed to Luminex Assay (use 50 µL per well)

Serum/Plasma

1. To 40 µL serum/plasma, add 10 µL of 1N HCL
2. Mix well
3. Incubate 10 minutes at RT.
4. Neutralize acidified sample by adding 8 µL of 1.2N NaOH/0.5M HEPES.
5. Mix well
6. Proceed to Luminex Assay (use 25 µL per well)

Warning:

When working with serum samples or serum standard diluent, carefully pipette the sample/diluents to avoid the creation of bubbles as this can reduce the performance of the Luminex assay.

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