

Sample Preparation for the Measurement of TGF beta 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 beta 1 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 beta 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 beta Sample Activation Procedure

Note: Do not activate kit standards.

Cell Culture Supernatants*

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

Serum/Plasma

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

*Pay attention to a possibly elevated blank signal in cell culture supernatant samples containing serum components (e.g. FCS), due to latent TGF beta levels in animal serum.

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