

Bio-Ocean LLC
3550 Lexington Ave, Suite 203
Shoreview, MN 55126
USA

Antigen retrieval protocol for human blood

The protocol uses acid and organic solution to retrieve antigens in protein-lipid complexes and make it easier for detection by ELISA method. The protocol was tested for some growth factors such as IGF-1, TGF. User will test their samples at their own risk and optimization is highly recommended.

1. Preparation of sample:

- I. For serum: let blood clot for 30 min at room temperature.
- II. For plasma, add EDTA to final concentration of 4.5 mM for 30 min. Then spin at 1000g for 15 min at room temperature.

2. Antigen retrieval:

In a 1.5 ml tube, mix 100ul of 2 N HCl with 800 ul of 100% ethanol. Then add 100ul of serum or plasma, vortex briefly, wait for 30min at room temperature

3. Neutralization of sample

Spin at 15000x g for 5 min, take out 250ul of cleared solution, add 50 ul of 1M NaOH, mix well, this is treated serum/plasma sample.

4. Dilution of sample

Add 200 ul of 1x sample dilution solution. The sample is now diluted 20 folds.

5. Assay of sample by ELISA

Add 100ul of diluted sample to each well in the 96-well plate. Perform ELISA assay following the instruction.