ELISA Extraction Methods from HemaSpot

Standard Curve Preparation

Standards are prepared in fresh whole blood, applied to HemaSpot or HemaForm plates and allowed to dry. Fresh whole blood should not contain the analyte of interest. Alternatively, PBS may be substituted for whole blood as long as extraction efficiencies have been determined to be equivalent.

1. Add analyte of interest to whole blood to achieve a target concentration high enough to exceed the concentrations expected to be seen in the samples.
   (Note: volume of analyte added to blood should not exceed 10% of the total volume. Care should be taken to minimize the use of organic solvents)

2. Serial dilute the highest standard with whole blood to create the remaining standards. Lowest standard should be less than the lowest value expected to be seen in the samples.
   (Note: limits of detection may determine the high and low standard concentrations)
   (Note: other methods of dilution are acceptable)

3. Apply 80 µL of each standard to HemaSpot (or HemaForm) and allow the samples to dry.

Extraction

4. Place 1-2 blades of each standard and sample into appropriately labeled microcentrifuge tubes or 96 well round bottom plates (standard or deep well).

5. Add 200 µL of extraction buffer.
   (Note: Volume of extraction buffer may vary from a minimum of 100 µL up to 300 µL)
   (Note: Extraction buffers are typically PBS +0.5% Tween or Triton-X. 1% BSA and protease inhibitors may be included for large protein extractions)

6. Shake samples gently.
   (Note: Extraction times are typically 5 to 60 minutes at room temperature for small molecules and easily extractable proteins. For large and/or sticky molecules, overnight (12-16 h) incubations at 2-8°C may be suitable)

7. Transfer extract to a clean tube or plate for analysis.
   (Note: Up to ~75% of original volume of extraction buffer can typically be recovered)
   (Note: Samples can be centrifuged to sediment paper if needed)

8. Perform analysis on extract according to ELISA kit recommended methods.
   (Note: Fluorescence and spectrophotometric methods (i.e. 450 nm) are generally suitable. However, possible interference from carryover of hemoglobin or other whole blood components should be determined during method evaluation)

For research use only. Not intended for use in diagnostic procedures.