**3.0 PRINCIPLE**

Immunoenzymometric assay (Type 3): The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay by spotting the enzyme-biotin on the surface of a microwell well through the interaction of streptavidin coated on the well and extensively added biotinylated monoclonal and Tropin-1 antibody.

Upon mixing biotin labeled monoclonal antibody, the enzyme-labeled antibody and a serum containing the native antigen results in a sandwich between the native antigen and the antibodies without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

\[
\text{ENZAb(x-GH)} - \text{AgGH} - \text{BtnAb(m)} = \text{Sandwich Complex}
\]

\[
\text{BtnAb(m)} = \text{Biotinylated Monoclonal Antibody (Excess Quantity)}
\]

**4.0 REAGENTS**

Materials Provided:

A. Growth Hormone Calibrators – 1ml/vial - Ions A-F
   Six (6) vials of references for hGH Antigen in human serum at levels of 0(A), 25(B), 50(C), 100(D), 150(E) and 200(F) µIU/ml.
   Store at 2-8°C. A preservative has been added.

Note: Calibrated against the 1st IS WHO 80/505. To convert to mass units in terms of the International Standard WHO 2nd IS# 98/574 divide by 3.7. WHO 80/505 (µU/ml) = 3.7 = WHO 98/574 (ng/ml).

B. hGH Tracer Reagent – 13µl/vial - Ions G-H
   One (1) vial contains horseradish peroxidase (HRP), labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Light Reaction Wells – 96 wells = Ions J
   One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Ions H
   One (1) vial contains a surfactant based solution buffered saline. A preservative has been added. Store at 2-8°C.

E. Signal Reagent A – 7.0ml/vial - Ions I
   One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Signal Reagent B – 7.0ml/vial - Ions I
   One (1) vial contains hydrogen peroxide (H₂O₂) dissolved in buffer. Store at 2-8°C.

G. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

**4.1 Required But Not Provided:**

1. Pipettes(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Luminometer
5. Container(s) for mixing of reagents (see below).
6. Vacuum aspirator (optional) for wash steps.
7. Plastic wrap or microplate cover for incubation steps.
8. Water bath (optional) for wash steps.
10. Storage container for storage of wash buffer.

**10.0 CALCULATION OF RESULTS**

A dose response curve is used to ascertain the concentration of hGH. Consider the following:

1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the percent intensity vs. duplicate reference serum concentration in µg/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hGH for an unknown, locate the appropriate percent intensity on the horizontal axis of the graph, find the intersecting point on the curve, and read the concentration (in µIU/ml) from the vertical axis of the graph. The concentration of the unknown serum may be determined by interpolation or extrapolation. In the following example, the average RLUs (20937) of patient intensities are plotted on the graph.

**Note:** Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
that may interfere in the assay and cause falsely low values. Genotypic variants are those that are not predicted according to the correlation-binding characteristics and affect final results. Such samples may display discordant results on different assays that utilized antibodies, which recognize different epitopes.

10. Accurate and precise pipetting, as well as following the exact protocol, is required to achieve reproducible results. Any deviation from the instructions may yield inaccurate results.

11. All applicable national standards, regulations, and laws, including, but not limited to, those established by the ATS, must be strictly followed to ensure compliance and proper device usage.

12. It is important to calibrate all equipment, e.g., Pipettes, Readers, Washers, and the automated instruments used with this device, and to perform routine preventative maintenance.

13. Risk Assessments and CE Mark IVD Directive 98/79/EC - this, and other devices, made by Monobind, can be requested from Monobind, Inc.

### 12.0 RISK ANALYSIS

#### 12.1 Assay Performance

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc. The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLU values of the calibrators have been normalized to 100% to facilitate RLU calculations. The conversion varies between instruments because of the efficiency of the various instruments that can be used to measure light output. This conversion eliminates differences in light output.

#### 12.2 Interpretation

Measurements and interpretation of results must be performed by a skilled individual or trained professional. Laboratory results should be interpreted with the appropriate context for determining what patient care should not be the sole basis for therapy. Particularly if the results conflict with other determinants.

#### 14.0 PERFORMANCE CHARACTERISTICS

### 14.1 Precision

This method was compared with a reference method: Biological specimens from normal and elevated samples were assayed. The results are depicted in Table 1. The precision of the method in the hands of the analyst. For these reasons each laboratory should determine the range of expected values and establish a protocol for reagent washes. Any deviation from Monobind IFU may yield inaccurate results. Any deviation from Monobind IFU may yield inaccurate results. Any deviation from Monobind IFU may yield inaccurate results. Any deviation from Monobind IFU may yield inaccurate results.

### 14.2 Sensitivity

14.4 Specificity

The cross-reactivity of the hGH AccuLite® CLIA test system to selected substances was evaluated by adding the interfering substance to the assay mix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Growth Hormone needed to produce the same absorbance.

### 15.0 REFERENCES


### 16.0 EFFECTED RANGES OF VALUES

Because of the pulsatile and sporadic nature of growth hormone secretion, reference intervals have little meaning. However, normal levels rarely have been reported for basal values are without significance. The failure of hGH to fall below 1µIU/ml within 60-120 minutes suggests excess hGH secretion.

It is important to keep in mind that establishment of a range of values for hGH can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specific population tested, the time of day the population tested, the precision of the method in the hands of the analyst. For these reasons each laboratory should determine the range of expected values and establish a protocol for reagent washes. Any deviation from Monobind IFU may yield inaccurate results. Any deviation from Monobind IFU may yield inaccurate results. Any deviation from Monobind IFU may yield inaccurate results. Any deviation from Monobind IFU may yield inaccurate results.

### 13.0 EXPECTED RANGES OF VALUES