Free Rat/Mouse IGF-I ELISA RUO

AL-136

INTENDED USE

The Free Rat/Mouse IGF-I enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IGF-I in rat and mouse serum, plasma, and other biological fluids.

SUMMARY AND EXPLANATION

Insulin-like growth factor I (IGF-I, a.k.a. somatomedin C) is a 7.6 kDa, 70 amino acid residue peptide, which mediates the actions of growth hormone (GH).[1] IGF-I is synthesized as a prohormone, a polypeptide consisting of A, C, B, D, and E domains.[1, 2] After post-translational modification, the mature IGF-I consist of the A, C, B and D domains, and is structurally homologous to IGF-II and insulin. In vivo, IGF-I is secreted by the liver and several other tissues and is postulated to have mitogenic and metabolic actions at or near the sites of synthesis; i.e. paracrine effects.[1] IGF-I also appears in the peripheral circulation where it circulates primarily in a high molecular weight tertiary complex with IGF-binding protein-3 (IGFBP-3) and acid-labile subunit (ALS).[2,3] A smaller proportion of IGF-I circulates in association with other IGF-binding proteins.[3]

Recently, there has been research interest in the measurement of serum/plasma "unbound" IGF-I which, theoretically, is the biologically active fraction. Although the existence of a true unbound IGF serum/plasma compartment is controversial, pharmacokinetic studies indicate that a small percentage of plasma IGF-I is not associated with IGF-binding proteins.[4,5] Unbound IGF-I has also been observed in saliva.[6] In addition, it appears that IGF-I may exert a tonic hypoglycemic effect under normal conditions that is inhibited by exogenous IGFBP-1 administration.[7]

It is likely that the measured unbound IGF-I fraction is a combination of the true unbound and the fraction of IGF-I that can be readily dissociated from IGFBP's under the specific assay conditions. In this respect, it has been shown that exogenously administered IGF-I almost immediately associates with low MW IGFBP's, then quickly moves into the high MW tertiary complex.[5,8,9] The tertiary complex does not appear to be easily dissociated and does not re-equilibrate with exogenously added IGF-I or IGFBP-3 to a significant degree.[8] On the other hand, the low MW complexes have a rapid turnover, and may be the source for much of the measured unbound IGF-I.

Various methods have been used to estimate the unbound (or freely dissociated) IGF-I fraction.[4,9,10] Size-exclusion chromatography and filtration methods.[4,9] have the theoretical disadvantage of altering the sample matrix and the equilibrium between IGF-I and IGFBP's. A direct detection unbound IGF-I assay using immobilized IGFBP-3 for capture and anti-IGF-I antibody for detection has been reported.[11]

The Ansh Labs Free Rat/Mouse IGF-I kit uses a highly sensitive two-site antibody method which allows detection of unbound IGF-I.

PRINCIPLE OF THE TEST

The Free Rat/Mouse IGF-I is a quantitative two-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples and biotinylated antibody solution are added to antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the second incubation and washing, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the mouse IGF-I antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of Mouse IGF-I in the samples and calibrators.

MATERIALS SUPPLIED

CAL-136A Free Mouse IGF-I Calibrators A / Sample Diluent

One bottle, 10 mL, labeled Free Mouse IGF-I Calibrator A/ Sample Diluent, containing 0 ng/mL IGF-I in buffer with Proclin 300. Store unopened at 2-8°C until the expiration date.

CAL-136B - CAL-136F Free Mouse IGF-I Calibrators B thru F

Five vials, 0.25 mL, labeled B-F, containing varying concentrations of Mouse IGF-I, in the range of 1.0 to 50 ng/mL (**Refer to Calibration Card for exact values**), in buffer with ProClin 300. Store unopened at 0 to -20°C until the expiration date. Avoid repeated freeze thaws.

NOTE: The calibrators are traceable to R&D Systems Mouse IGF-I preparation (Catalog number 791-MG-050).

CTR-136-I & CTR-136-II Free Mouse IGF-I Controls I & II

Two vials, 0.25 mL, labeled Levels I and II containing low and high IGF-I concentrations (**Refer to Calibration Card for exact values**) in buffer with ProClin 300. Store unopened at 0 to -20°C until the expiration date. Avoid repeated freeze thaws.

PLT-136 Rat/Mouse IGF-I Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with IGF-I antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

CND-136 Mouse IGF-I Conjugate Diluent

One bottle, 12 mL, containing a protein-based (BSA)-buffer with non-mercury preservative. Store at 2-8°C until the expiration date.

BCC-136 Free Mouse IGF-I Biotin Conjugate Concentrate (50X)

One vial, 0.4 mL containing biotinylated detection antibody in a protein-based buffer with a non-mercury preservative. Dilute prior to use in Total Mouse IGF-I assay. Store at 2-8°C until expiration date

SAR-137 Free Mouse IGF-I Streptavidin-Enzyme Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 5–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

WARNING: Potential Biohazardous Material

Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[12].

WARNING: Potential Chemical Hazard

Some reagents in this kit may contain Proclin 300 and Sodium azide[13] as a preservative. Proclin 300 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Serum is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples should be stored frozen at -20°C or lower.

d) Avoid repeated freezing and thawing of samples.

e) Avoid assaying lipemic, hemolyzed or icteric samples.

f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Free Rat/Mouse IGF-I ELISA assay. It is the customer's responsibility to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
2. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.
3. **Free Mouse IGF-I Antibody-Biotin Conjugate Solution**: The Free Mouse IGF-I Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of Mouse IGF-I Conjugate Diluent, according to the number of wells used. If an entire plate is to be used pipet exactly 220µL of the Concentrate into 11 mL of the buffer.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate. *Specimens reading higher than the last calibrator should be diluted with sample diluent.*

Note: All Rat samples should be diluted 1:20, and all mouse samples should be diluted 1:10 prior to assay.

1. Label the microtitration strips to be used.
2. **Sample Preparation:**
   a) For Rat Samples: 1:20 dilution
      i) For each unknown rat sample, label one eppendorf vial appropriately and add 47.5 µL of Calibrator A/Sample Diluent to each vial.
      ii) Add 2.5 µL of the rat specimens to the pre-labeled vials and vortex well.
   b) For Mouse Samples: 1:10 dilution
      i) For each unknown mouse sample, label one eppendorf vial appropriately and add 45 µL of Calibrator A/Sample Diluent to each vial.

ii) Add 5 µL of the mouse specimens to the pre-labeled vials and vortex well.

3. Pipette **10 µL** each of the **Calibrator, Controls and Unknowns** to the appropriate wells.
4. Add **100 µL** of the **Antibody-Biotin Conjugate** to each well using a repeater pipette. (Prepare the Free Mouse IGF-I Antibody-Biotin Conjugate Solution by diluting the Free Mouse IGF-I Biotin Conjugate Concentrate in Mouse IGF-I Conjugate Diluent as described under the Preparation of the Reagents section of this package insert.)
5. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
6. Aspirate and wash each strip **5 times** with Wash Solution **(350 µL/per well)** using an automatic microplate washer.
7. Add **100 µL** of the **Free Mouse IGF-I Streptavidin-Enzyme Conjugate-RTU** to each well using a repeater pipette.
8. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
9. Aspirate and wash each strip **5 times** with Wash Solution (350 **µL/per well)** using an automatic microplate washer.
10. Add **100 µL** of the **TMB chromogen solution** to each well using a repeater pipette. Avoid exposure to direct sunlight.
11. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    ***NOTE**: Visually monitor the color development to optimize the incubation time.*
12. Add **100 µL** of the **Stopping solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.

***NOTE**: Zero calibrator should be programmed as "**Blank**" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm**.*

RESULTS

***NOTE:** The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log IGF-I concentration on X-axis** using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the IGF-I concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the IGF-I concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding IGF-I concentrations.
4. Any sample reading lower than the analytical sensitivity should be used as measured concentration.
5. Multiply the measured concentration in ng/mL by the dilution factor (Step 2).

LIMITATIONS

The reagents supplied in this kit are optimized to measure rat/mouse IGF-I levels in serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophilic antibodies in the samples.[14]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Free Rat/ Mouse IGF-I ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Free Mouse IGF-I controls are printed on the Calibration card.
- A full calibration curve, low- and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Calibrators	Mean Absorbance	Conc. (ng/mL)
A1, A2	A	0.081 (**Blank**)	0
B1, B2	B	0.031	1.0
C1, C2	C	0.126	3.0
D1, D2	D	0.526	9.0
E1, E2	E	1.741	25.0
F1, F2	F	3.212	50.0

***CAUTION**: The above data must not be employed in lieu of data obtained by the user in the laboratory*

ANALYTICAL CHARACTERISTICS

All concentrations listed are in ng/mL.

Analytical Sensitivity

The analytical sensitivity in the Free Rat/Mouse IGF-I assay, as calculated by the interpolation of mean plus two standard deviations of 16 replicates of Calibrator A (0 ng/mL) and Calibrator B (1.0 ng/mL), is **0.81 ng/mL**.

Imprecision

Reproducibility of the Free Rat/Mouse IGF-I assay was determined in a study using three controls. The study included a total of 5 assays, 3 replicates of each per assay (n=15). Representative data were calculated based on EP10A-3 guidelines and are presented in the following table.

Sample	Mean Conc. (ng/mL)	SD	%CV
1	2.8	0.214	7.72
2	6.6	0.32	4.82
3	12.7	0.76	6.00

Linearity of Dilution

Calibrator F containing various mouse IGF-I levels was diluted serially in Calibrator A/Sample Diluent. The % recovery is presented in the following table.

Sample ID	Dilution factor (1 in X)	Expected Value in ng/mL	Observed Value in ng/mL	%Recovery	Average %Recovery
CAL-F	NEAT VALUE	50.000			96%
	2	25.000	24.264	97%	
	4	12.500	12.376	99%	
	8	6.250	5.830	93%	
	16	3.125	2.759	88%	
	32	1.563	1.576	101%	

Analytical Specificity

Cross-reactivity to other closely related analyte is represented in the table below.

Sample No.	Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
1	Mouse IGFBP-2	50	ND
2	Mouse IGFBP-3	50	ND
3	Mouse IGFBP-4	50	ND

4	Mouse IGFBP-5	50	ND
5	Mouse IGF-II	50	ND
6	Rat IGF-II	50	ND

Species Immunoreactivity

The monoclonal antibody pair used in the assay detects **bovine, equine, canine, rabbit, goat, squirrel, porcine, rat, ovine, and monkey** as represented in table below.

Sample#	Species	Type	Dilution Factor	O.D.	Observed Conc. (ng/mL)	Calculated Conc. (ng/mL)
1	Rabbit	Serum	1	1.892	15.518	15.518
2	Rabbit	Serum	1	3.266	>24.033	>24.033
3	Rabbit	Serum	1	3.836	>24.033	>24.033
4	Goat	Serum	1	0.264	3.274	3.274
5	Goat	Serum	1	0.035	ND	ND
6	Goat	Serum	1	0.28	3.479	3.479
7	Bovine	Serum	1	0.345	4.235	4.235
8	Bovine	Serum	1	0.114	ND	ND
9	Bovine	Serum	1	1.016	9.714	9.714
10	Canine	Serum	1	0.171	1.801	1.801
11	Canine	Serum	1	0.111	ND	ND
12	Canine	Serum	1	0.175	1.882	1.882
13	Equine	Serum	1	0.135	ND	ND
14	Equine	Serum	1	0.15	1.309	1.309
15	Equine	Serum	1	0.383	4.636	4.636
16	Feline	Serum	1	0.047	ND	ND
17	Feline	Serum	1	0.052	ND	ND
18	Ovine	Serum	1	0.315	3.899	3.899
19	Ovine	Serum	1	0.065	ND	ND
20	Porcine	Serum	1	0.277	3.441	3.441
21	Porcine	Serum	1	0.234	2.863	2.863
22	Mouse	Serum	10	0.921	9.046	90.46
23	Mouse	Serum	10	1.246	11.277	112.77
24	Mouse	Serum	10	1.713	14.345	143.45
25	Rat	Serum	10	2.828	22.024	220.24
26	Rat	Serum	10	3.048	23.728	237.28
27	Rat	Serum	10	2.546	19.962	199.62
28	Squirrel Monkey	Serum	1	0.144	1.128	1.128
29	Vervet Monkey	Serum	1	3.659	>24.033	>24.033

ND-Non-Detectable

Interference

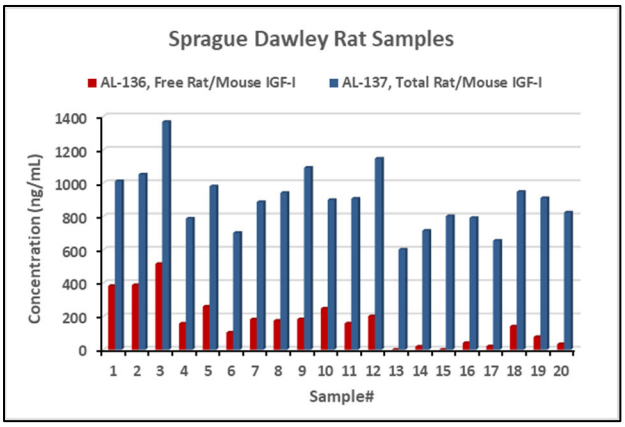
When hemoglobin, biotin, intralipids and bilirubin were added at greater than two folds of their physiological concentrations to control samples, average mouse IGF-I concentrations were within ± 15% of the control as represented in the following table.

Interferent	Interferent Dose	Sample IGF-I (ng/mL)	Dosed Sample IGF-I (ng/mL)	% Difference
Hemoglobin	0.5 mg/mL	17.16	16.86	-1.8
	0.5 mg/mL	2.92	3.03	3.6
Biotin	1200 ng/mL	16.41	17.02	3.7
	600 ng/mL	17.58	17.08	-2.8
Biotin	1200 ng/mL	2.74	2.72	-0.6
	600 ng/mL	3.07	2.97	-3.4
Intralipids	20 mg/mL	3.28	2.89	-12.0
	10 mg/mL	3.09	2.85	-7.7
Bilirubin	0.2 mg/mL	6.88	6.99	1.5
	0.2 mg/mL	3.10	3.47	12.0

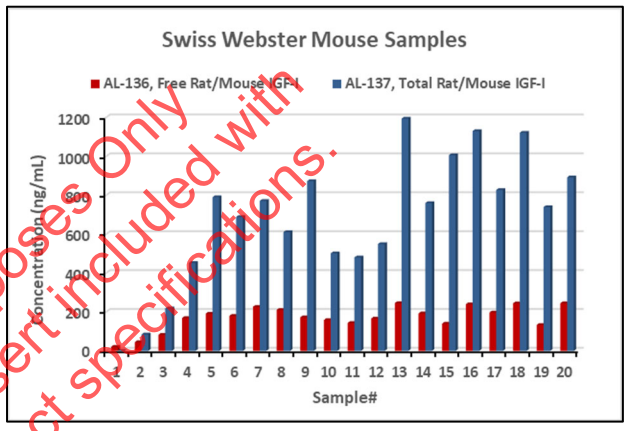
Total to Free IGF-I Concentration

20 Sprague Dawley rat samples and 20 Swiss Webster mouse samples were assayed in Ansh Labs Free Rat/Mouse IGF-I ELISA (AL-136) and Total Rat/Mouse IGF-I ELISA (AL-137).

The % average concentration of Free IGF-I to Total IGF-I in Sprague Dawley rat samples was calculated to be 18% as represented in figure below.



The % average concentration of Free IGF-I to Total IGF-I in Swiss Webster mouse samples was calculated to be 28% as represented in figure below.



REFERENCES

1. Daughaday E, Rotwein P: Insulin like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum and tissue concentrations. Endocrin Rev 10:68-91, 1989.
2. Baxter RC, Martin JL, Beniac VA: High molecular weight insulin-like growth factor binding protein complex. J Biol Chem 264:11843-11848, 1989.
3. Rechler M: Insulin-like growth factor binding proteins. Vit Horm 47:1-114, 1993.
4. Guler HP, Zapf J, Froesch ER: Short-term metabolic effects of recombinant human insulin-like growth factor-I in healthy adults. New Engl J Med 317:137-140, 1987.
5. Zapf J, Hauri C, Waldvogel M, Froesch ER: Acute metabolic effects and half-lives of intravenously administered insulinlike growth factors I and II in normal and hypophysectomized rats. J Clin Invest 77:1768-1775, 1986.
6. Costigan DC, Guyda HJ, Posner BI: Free insulin-like growth factor I (IGF-I) and IGF-II in human saliva. J Clin Endocrinol Metab 66:1014-1018, 1988.
7. Lewitt MS, Denyer GS, Cooney GJ, Baxter RC: Insulin-like growth factor-binding protein-1 modulates blood glucose levels. Endocrinology 129:2254-2256, 1991.
8. Lewitt MS, Saunders H, Baxter RC: Bioavailability of insulin-like growth factors (IGFs) in rats determined by the molecular distribution of human IGF-binding protein-3. Endocrinology 133:1797-1802, 1993.
9. Lieberman SA, Bukar J, Chen SA, Celniker AC, Compton PG, Cook J, Albu J, Perlman AJ, Hoffman AR: Effects of recombinant human insulin-like growth factor-I (rhIGF-I) on total and free IGF-I concentrations, IGF-

binding proteins, and glycemic response in humans. J Clin Endocrinol Metab 75:30-36, 1992.

10. Lee PDK, Powell D, Baker B, Liu F, Mathew G, Levitsky I, Gutierrez OD, Hintz RL: Characterization of a direct, non-extraction immunoradiometric assay for free IGF-I. Presented at the 76th annual meeting of the Endocrine Society, Anaheim, 1994.
11. Mukku VR: A 96-well microtiter plate assay for free IGF-I in plasma using immobilized IGFBP-3. Presented at the 73rd annual meeting of The Endocrine Society, Washington, D.C., 1991. (abstract #462).
12. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
13. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.
14. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

Manufactured by:
Ansh Labs
445 Medical Center Blvd.