

# EDI™ Human Chromogranin A ELISA Kit

Enzyme Linked Immunosorbent Assay (ELISA) for the measurement of Human Chromogranin A Level

REF KTR-820 RUO 

## INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human chromogranin A levels in EDTA-plasma and serum samples. This assay exclusively measures human chromogranin A without the high dose "hook" effect up to 1,000,000 ng/mL. This test may be used as an aid for detecting patients with pheochromocytoma and neuroendocrine tumors (carcinoids). This kit is for research use only.

## SUMMARY OF PHYSIOLOGY

Chromogranin A is a 49 kDa acidic protein that consists of 439 amino acids encoded on chromosome 14. Chromogranin A has been identified in a number of normal and neoplastic endocrine tissues. It is demonstrated that an elevated level of circulating chromogranin A is a marker for tumors of neuroendocrine origin. However, the most significant clinical use of chromogranin A is related to the diagnostic procedure in patients with pheochromocytoma. The following is a short summary of the potential usages of chromogranin A.

1. A very sensitive (83%) and highly specific (96%) marker in the evaluation of actual or suspected pheochromocytoma. Drugs commonly employed in the diagnosis or treatment of pheochromocytoma have little effect on the plasma chromogranin A level, which is a great advantage of measuring chromogranin A over catecholamines.
2. To ascertain the source of a tumor. A high chromogranin A level indicates that the tumor arises from neuroendocrine tissues.
3. Endocrine tumors that do not produce their specific hormones, for example, calcitonin negative but chromogranin A positive C-cell carcinoma; zero-cell carcinoma; beta-cell carcinoma; parathyroid carcinoma.

## ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human chromogranin A in EDTA-plasma or serum sample. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human chromogranin A.

Assay calibrators, controls and patient samples are added directly to wells of microplate that is coated with a polyclonal chromogranin A antibody. After the first incubation period, the antibody on the wall of microtiter well captures human chromogranin A in the sample and unbound protein in each microtiter well is washed away. Then a horseradish peroxidase (HRP)-labeled monoclonal anti-human chromogranin A antibody is added to each microtiter well and a "sandwich" of "monoclonal antibody - human chromogranin A - polyclonal antibody" is formed. The unbound monoclonal antibody is removed in the subsequent washing step. For the detection of this immunocomplex, the well is incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the chromogranin A on the wall of the microtiter well is directly proportional to the amount of chromogranin A in the sample. A calibration curve is generated by plotting the absorbance versus the respective human

chromogranin A concentration for each calibrator with a 4 parameter curve fit. The concentration of human chromogranin A in test samples is determined directly from this calibration curve.

## REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

### 1. Anti-CgA Antibody Coated Microplate (30063)

Microplate coated with human chromogranin A antibody.

Qty: 1 x 96 well microplate

Storage: 2 – 8°C

Preparation: Ready to Use

### 2. CgA Tracer Antibody (30431)

HRP-labeled anti-human chromogranin A monoclonal antibody in a stabilized protein matrix.

Qty: 1 x 12 mL

Storage: 2 – 8°C

Preparation: Ready to Use

### 3. CgA Assay Buffer (30074)

Phosphate-buffered saline based buffer with bovine serum albumin added.

Qty: 1 x 30 mL

Storage: 2 – 8°C

Preparation: Ready to Use

### 4. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL

Storage: 2 – 8°C

Preparation: Ready to Use

### 5. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL

Storage: 2 – 25°C

Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

### 6. ELISA Stop Solution (10030)

0.5 M sulfuric acid.

Qty: 1 x 12 mL

Storage: 2 – 25°C

Preparation: Ready to Use

## 7. Chromogranin A Calibrators Levels 1 to 5 (30064-30068)

Human chromogranin A in a lyophilized bovine serum albumin-based matrix with a proclin preservative. Refer to each vial for exact concentration.

Qty: 5 x Vials

Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)

Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

## 8. Chromogranin A Controls (30069,30070)

Human chromogranin A in a lyophilized bovine serum albumin-based matrix with a proclin preservative. Refer to each vial for exact concentration.

Qty: 2 x Vials

Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)

Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

## SAFETY PRECAUTIONS

The reagents are for research use only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µL, 65 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with cap.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450/650 nm or 450/620 nm..

## SPECIMEN COLLECTION & STORAGE

Only 30 µL total (15 µL each) of human EDTA-plasma or serum is required for human chromogranin A measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected with lavender-top Vacutainer. Separate the plasma from cells by centrifugation (850 – 1500xg for 10 minutes). The plasma should be separated from the cells within one hour of blood collection and transferred to a clean test tube. **Plasma samples should be stored below – 15°C** if the assay is not to be performed within 72 hours. Otherwise, the plasma samples should be stored at room temperature for up to 72 hours. It is important that the plasma samples **must not** be stored at 2 –8°C. Avoid more than three freeze-thaw cycles of specimen.

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Serum sample can also be used for chromogranin A measurement. Tests with paired EDTA-plasma and serum sample from same donor shows that serum gives almost the same chromogranin A level as EDTA-plasma by using this ELISA.

Collected EDTA-plasma or serum samples should be shipped to designated laboratory in frozen condition with dry ice. In case frozen condition is not available, samples should be shipped at room temperature in an insulated container for maximum 48 hour delivery. Samples must **not** be shipped refrigerated, such as, with blue ice pack.

## ASSAY PROCEDURE

### 1. Reagent Preparation

1. Prior to use allow all reagents to come to room temperature (20-25 °C). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Reconstitute all assay calibrators (30064-30068) and controls (30069-30070) by adding 0.5 mL of demineralized water to each vial. Allow the calibrators and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use. These reconstituted calibrators and controls must be stored at -20°C or below. Do not exceed 3 freeze-thaw cycles.

### 2. Manual Assay Procedure

1. Place a sufficient number of microwell strips (30063) in a holder to run calibrators (30064 - 30068), controls (30069,30070), and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add **15 µL** of calibrators (30064 - 30068), controls (30069,30070), and samples into the designated microwells.
4. Add **200 µL** of assay buffer (30074) into each microwell.
5. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 °C)** with **shaking at 350 to 450 rpm for 60 minutes**.
6. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
7. Add **100 µL** of CgA Tracer Antibody (30431) to each well. Mix by gently tapping the plate.

8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 °C)** with **shaking at 350 to 450 rpm for 60 minutes**.
9. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
10. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 °C)** for **20 minutes**.
12. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
13. Read the absorbance at **450/650 nm** or **450/620 nm** with a **4-parameter curve fit** within **10 minutes** with a microplate reader.

### 3. Automated Assay Procedure (Dynex DS-2)

1. Load a sufficient number of antibody coated microwell strips (30063) onto the system to run human chromogranin A standards, controls and unknown samples in duplicate.
2. Load sufficient Chromogranin A Tracer antibody (30431).
3. Prepare and load kit calibrators (30064 – 30068), controls (30069, 30070), samples, substrate (10020), Stop Solution (10030), and diluted Wash Buffer (10010) onto the system accordingly.
4. Add **200 µL** of assay buffer (30074) to each well
5. Add **15 µL** of calibrators, controls and samples into the designated microwells.
6. Incubate plate with initial shaking for **1 minute** and then at room temperature for **60 minutes**.
7. Wash each well **4 - 5 times**.
8. Add **100 µL** of tracer antibody (30431) to each of the wells.
9. Incubate plate at **room temperature (20-25 °C)** for **60 minutes**.
10. Wash each well **4 - 5 times**.
11. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells.
12. Incubate plate at **room temperature (20-25 °C)** for **15 - 20 minutes**.
13. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix gently.
14. Read the absorbance at **450/650 nm** or **450/620 nm** with a 4-parameter curve fit program.

*Note: Open automated ELISA system other than DS-2 can also be used. This may require adjustment of the protocol. It is very important to incubate the assay 20-25°C. A change of incubation temperature would cause unsatisfactory calibration curve and erroneous test results.*

### PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. If a Tecan is used for pipetting, it is recommended to add 200 µL assay buffer before adding the 15 µL assay calibrators, controls and test samples into each designated well. This is the same as the procedure with DS-2, but a reverse with the manual procedure.
3. Keep light-sensitive reagents in the original amber bottles.
4. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.

7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.
9. We strongly recommend using 4-Parameter curve fit for control and patient sample calculation. Other curve fit programs such as Point-to-Point, Log-Log, Log-Linear, etc. may give a poor linear recovery.

### INTERPRETION OF RESULTS

The human chromogranin A concentrations for the controls and patient samples are read directly from the calibration curve using their respective corrected absorbance.

Laboratory should report test results directly derived from the assay. For samples showing a higher than 90% value of the highest assay calibrator, it is strongly recommended that the patient sample is diluted 1:100 with assay buffer and re-assay the diluted sample for a more accurate test result. For example, the highest assay calibrator is about 550 ng/ml, any sample that shows a value of greater than 500 ng/ml (90% of 550 ng/ml) should be repeated with a 1:100 diluted sample. If the 1:100 diluted sample still shows a higher value than that of the highest assay calibrator, one can either report the sample value as greater than the highest assay calibrator (e.g. > 56,000 ng/ml) or further measure a 1:10,000 diluted sample. It is preferred to obtain a diluted sample value located between calibrator level 2 and level 4, wherein, this measured value is multiplied by the dilution factor to obtain the report value for the patient.

### LIMITATIONS OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for human chromogranin A measurement, the values of assay calibrators were established by correlation to a highly purified chromogranin A calibrator.
2. For sample values reading greater than the highest calibrator or 90% value of the highest calibrator, it is recommended to re-assay samples with dilution.
3. Storing samples at refrigerated condition causes significant degradation of intact chromogranin A into small fragments. These fragments may cause interference of the assay resulting in false low test result.
4. Serum samples are not as stable as EDTA-plasma samples. Therefore, it is strongly recommended to use EDTA-plasma sample for chromogranin A measurement.
5. Bacterial or fungal contamination of plasma specimens or reagents, or cross-contamination between reagents may cause erroneous results.
6. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

### QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known chromogranin A levels. We recommend that all assays include the laboratory's own chromogranin A controls in addition to those provided with this kit.

### EXPECTED VALUES

Seventy-two normal adult sera were measured with this human chromogranin A ELISA. The normal range was found to be less than 100 ng/mL. Five patients with pheochromocytoma showed a chromogranin A level of significantly over 100 ng/ml and one of them reached 400,000 ng/mL. It is highly recommended that each laboratory establish its own normal cut-off level. Paired EDTA-Plasma and Serum samples give almost the same values.

Although a plasma or serum chromogranin A level above 100 ng/ml would be an aid in clinical diagnosis, it is recommended to establish a baseline level of chromogranin A for each patient in order to monitor cancer patients after surgery, especially if this assay is used for the

monitoring of prostate cancer patients. A clear surge of chromogranin A level would indicating an increased cancer cell activity.

Some endocrine diseases such as primary hyperparathyroidism, hyperthyroidism, or secondary hyperparathyroidism caused by chronic renal failure would also give a higher than normal serum chromogranin A level. It is reported that patients with rheumatoid arthritis, Systemic Lupus Erythematosus would also cause higher chromogranin A level. Some therapeutic drugs that stimulate the endocrine system such as sexual hormone releasing hormone may also cause higher chromogranin A level in patient sample.

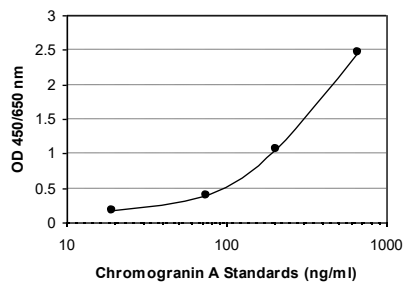
EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from human chromogranin A ELISA are represented.

Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450/650 nm)			Concentration (ng/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 ng/mL	0.107	0.110	0.000	
	0.112			
Calibrator Level 2: 19.2 ng/mL	0.185	0.184	0.074	
	0.184			
Calibrator Level 3: 75 ng/mL	0.400	0.400	0.290	
	0.400			
Calibrator Level 4: 203 ng/mL	1.098	1.064	0.954	
	1.031			
Calibrator Level 5: 660 ng/mL	2.442	2.465	2.355	
	2.488			
Control 1	0.248	0.248	0.138	40.66
	0.248			
Control 2	0.452	0.456	0.346	84.51
	0.461			

Human Chromogranin A ELISA



PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity (LLOD or LOD) of the human chromogranin A ELISA is approximately 2 ng/mL, which is determined by 2 standard deviations above the average of zero calibrator in 7 replicate determination of zero calibrator and 7 replicate determination of calibrator level 2 of 19.2 ng/mL.

Hook Effect

This assay has showed that it did not exhibit any high dose “hook” effect up to 1,000,000 ng/mL. This is essential because some patients with KTR-820/V14/RUO/2024-02

pheochromocytoma had over 300,000 ng/mL of chromogranin A level in their serum sample.

Specificity

The assay was performed to assess the cross-reactivity and results are summarized in below table:

Cross-reactants	Result
ACTH	No cross-reactivity was found
FGF-21	
Osteocalcin	
Hemoglobin	
Liquids	

Reproducibility and Precision

The intra-assay precision is validated by measuring two controls samples in a single assay with 8 replicate determinations. The inter-assay precision is validated by measuring two control samples in duplicate in 12 individual assays. The results are as follows:

Sample	Intra-Assay		Inter-Assay	
	1	2	1	2
Mean (ng/mL)	28.24	59.81	41.91	82.74
CV (%)	2.9	3.7	7.3	3.1

Linearity

Three human serum samples were diluted with assay buffer and assayed. The results are as follows:

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample A	717.5	-	-
50%	357.4	358.8	100
25%	171.1	179.4	95
12.5%	74.6	89.7	83
Sample B	932.7	-	-
50%	495.1	466.4	106
25%	229.3	233.2	98
12.5%	112.2	116.6	96
6.25%	54.5	58.3	93
Sample C	467.8	-	-
50%	234.6	233.9	100
25%	117.8	117.0	100
12.5%	52.2	58.5	89

Spike Recovery

Three patient serum samples were spiked with various amounts of human chromogranin A control samples and assayed. The results indicate below:

Samples	Observed (ng/mL)	% Recovery
Sample A	12.78	-
Level 3	62.43	-
25% Sample A + 75% Level 3	46.48	93
50% Sample A + 50% Level 3	37.94	100
75% Sample A + 25% Level 3	24.27	95
Sample B	51.74	-
Level 4	185.74	-
25% Sample B + 75% Level 4	143.91	95
50% Sample B + 50% Level 4	112.07	121
75% Sample B + 25% Level 4	80.22	94
Sample C	73.00	-
Level 4	185.74	-
25% Sample C + 75% Level 4	149.27	95
50% Sample C + 50% Level 4	125.61	97
75% Sample C + 25% Level 4	91.72	91

Interference

Bilirubin, hemoglobin and lipid triglycerides were tested as potential interferents to the human chromogranin A ELISA kit. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:  
It is indicated that hemolyzed sample may not be suitable for chromogranin A measurement.

Interferent (Concentration tested, mg/mL)	Test (ng/mL)	Control (ng/mL)	Bias ( $d_{obs}$ , %)	
Bilirubin (EP07 recommended concentration: 0.05 mg/mL)	2.0	228.6	259.7	12.0%
	0.2	235.8	259.7	9.2%
	0.04	244.3	259.7	5.9%
Hemoglobin (EP07 recommended concentration: 2 mg/mL)	20.0	184.0	259.7	29.1%
	2.0	240.8	259.7	7.3%
	0.4	260.8	259.7	0.4%
Lipids (EP07 recommended concentration: 5 mg/mL)	400.0	55.6	58.4	4.8%
	40.0	59.0	58.4	1.0%
	20.0	56.2	58.4	3.8%
	6.0	258.9	256.7	0.3%

### WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

### REFERENCES

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### TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

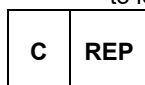
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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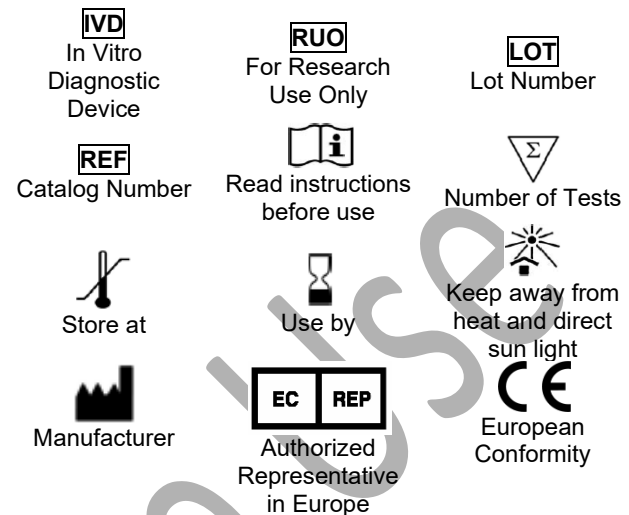
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### GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



### SHORT ASSAY PROCEDURE

#### 1. Manual Assay Procedure

- Add **15 µL** of the calibrators, controls, and samples into the designated microwells.
- Add **200 µL** of the assay buffer to each well.
- Mix, cover, and incubate at **room temperature (20-25 °C)** with **shaking 350 rpm – 450 rpm** for **60 minutes**.
- Wash each well five times.
- Add **100 µL** of the tracer antibody to each well.
- Cover and incubate at **room temperature (20-25 °C)** with **shaking 350 rpm – 450 rpm** for **60 minutes**.
- Wash each well five times
- Add **100 µL** of substrate to each well.
- Cover and incubate at **room temperature (20-25 °C)** for **20 minutes**.
- Add **100 µL** of the stop solution to each well.
- Read the absorbance at **450/650 nm** or **450/620 nm**.

#### 2. Automated Assay Procedure

- Prepare and load reagents.
- Add **200 µL** of assay buffer to each well.
- Add **15 µL** of calibrators, controls and samples into the designated microwells.
- Incubate plate with initial shaking for **1 minute** and then at room temperature for **60 minutes**.
- Wash each well **4 - 5 times**.
- Add **100 µL** of tracer antibody to each well.
- Incubate plate at **room temperature (20-25 °C)** for **60 minutes**.
- Wash each well **4 - 5 times**.
- Add **100 µL** of ELISA HRP Substrate to each well.
- Incubate plate at **room temperature (20-25 °C)** for **15 - 20 minutes**.
- Add **100 µL** of stop solution to each well
- Read the absorbance at **450/650 nm** or **450/620 nm**.