Oocyte Maturation Quality Check (OMQ) Inhibin-A ELISA

RUO

AL-1009-r

INTENDED USE

The Oocyte Maturation Quality (OMQ) Check enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of dimeric Inhibin A in human serum. This assay is intended for research use only.

SUMMARY AND EXPLANATION

Inhibins are heterodimeric protein hormones secreted by granulosa cells of the ovary in the female and Sertoli cells of the testis in the male. They selectively suppress the secretion of pituitary follicle stimulating hormone (FSH) and have local paracrine actions in the gonads. The fully processed form of the inhibin molecule has a molecular weight of approximately 32 kD and consists of the two distinct chains (α and β), linked by disulfide bridges. Higher molecular weight forms, with precursor forms of the α-subunit, also occur in follicular fluid and serum. In addition, free α-subunit forms, unassociated with a β-subunit, and lacking inhibin bioactivity, are also present. Inhibin A consists of an α-subunit and β_A-subunit.

Recently, Inhibin-A and Inhibin-B are emerging as markers for the assessment of developing follicles during ovarian stimulation[1] and can assist with the follicle dynamics. The number of mature oocytes is a key factor in the IVF success. Gonadotropin dosing during ovarian stimulation cycle is a critical step in maximizing the oocytes yield. The serum estradiol and transvaginal ultrasound measurements are currently used as monitoring tools in stimulated cycle. Serum Estradiol levels are commonly supraphysiological and often variable due to multi-follicular growth of varying size of follicles in IVF cycles. Additionally, Estradiol binds to peripheral SHBG and the competitive nature of the immunoassay makes Estradiol measurement unreliable for checking the oocyte maturation quality. In contrast, serum Inhibin A levels increase only when follicle size are between 12–15 mm. This makes serum Inhibin-A a better marker for studying the Oocyte quality. Along with routine ultrasound measurements, Inhibin A has been studied for optimal timing of trigger medication with a threshold of ≥10 mature oocytes.[1] Serum Inhibin-A levels strongly correlate to the number of follicles ≥15mm and mature oocytes. Measurements of Inhibin-A are shown to be useful in studying its role in human reproductive physiology.[1]

PRINCIPLE OF THE TEST

The OMQ Check ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to Inhibin-A antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated Inhibin A antibody. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the 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 Inhibin A in the samples and calibrators.

MATERIALS SUPPLIED

CAL-1009A Inhibin A Calibrators A

One vial, 6 mL, labeled A, containing 0 pg/mL Inhibin A in animal sera and a non-mercury preservative. Store unopened at 2-8°C until the expiration date. Discard after 5 days, if stored at 2-8°C. For longer storage, aliquot and freeze at -20°C or colder for up to one year.

CAL-1009B-CAL-1009F Inhibin A Calibrators B thru F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 41-6000 pg/mL Inhibin A in animal sera and a non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2-8°C until the expiration date. Reconstitute calibrators B-F with **1.0 mL** deionized water. Solubilize for **10 minutes**, mix well, and use after reconstitution. Discard after 5 days, if stored at 2-8°C. For longer storage after reconstitution, aliquot and freeze at -20°C or colder for up to one year.

STANDARDIZATION NOTE: *The Inhibin A calibrators are traceable to the World Health Organization International Preparation Inhibin A NIBSC code 91/624 version 3.0, Dated 01/25/2008*

CTR-1009-I & CTR-1009-II Inhibin A Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high Inhibin A concentrations in animal sera and a non-mercury preservative. Refer to **calibration card** for exact control ranges. Store unopened at 2-8°C until the expiration date. Reconstitute control Levels I and II with **1.0 mL** deionized water. Solubilize for **10 minutes**, mix well, and use after reconstitution. Discard after 5 days, if stored at 2-8°C. For longer storage after reconstitution, aliquot and freeze at -20°C or colder for up to one year.

PLT-123 Inhibin A Coated Microtitration Strips

One stripholder, containing 12 strips and 96 microtitration wells with Inhibin A 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.

ASB-123A Inhibin A Assay Buffer A

One bottle, 6 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

ASB-123B Inhibin A Buffer B

One bottle, 6 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

BCR-1009 OMQ Check Biotin Conjugate Ready-to-Use (RTU)

One bottle, 12 mL, containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

SAR-1009 OMQ Check 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 2-30°C until expiration date.

WSH-100 Wash Concentrate A

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

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of absorbance measurement at 450 nm, 405 nm, and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.

WARNINGS AND PRECAUTIONS

For *in vitro research* use only.

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

This reagent may contain some human source material (e.g., serum) or materials used in conjunction with human source materials. 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[2].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[3] as a preservative. Pro-Clean 400 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 SDS, 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 may be stored at 4°C if assayed within 24 hours; otherwise, samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

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[4].

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the OMQ Check ELISA assay. It is the user's responsibility to validate the assay for their purpose. 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 (23 ± 2°C) 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. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the substrate solution into the wells. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

PREPARATION OF REAGENTS

1. **Inhibin A Calibrators B-F and Inhibin A Controls I & II**: Tap and reconstitute Inhibin A Calibrators B-F and Inhibin A Controls I & II each with **1.0** mL deionized water. Solubilize for **10 minutes**, mix well, and use after reconstitution.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (23 ± 2°C) when stored in a tightly sealed bottle.
3. **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.
4. **Inhibin A Assay Buffers Premix Solution:** The Inhibin A Assay Buffer A (ASB-123A) and Inhibin A Assay Buffer B (ASB-123B) should be mixed by gentle inversion in equal volumes (1:1 ratio) according to the number of wells used. If an entire plate is to be used mix exactly 3 mL of the ASB-123 B into 3 mL of the ASB-123 A. The pre-mixture solution is stable for use up to 4 hours. Discard the pre-mix solution after 4 hours.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

NOTE: *All serum samples reading higher than the highest calibrator should be mixed and diluted in the 0 pg/mL Calibrator A prior to assay.*

1. Reconstitute Inhibin A Calibrators B-F with **1.0 mL** deionized water. Solubilize for **10 minutes**, Mix well.
2. Reconstitute Inhibin A Controls I & II each with **1.0 mL** deionized water. Solubilize for **10 minutes**, Mix well.
3. Label the microtitration strips to be used.
4. Pipette **50 µL** of the Calibrator, Controls and Unknowns to the appropriate wells.
5. Add **50 µL** of Inhibin A Assay Buffers Premix Solution (ASB-123A and ASB-123B in 1:1 ratio as described under the Preparation of the Reagents section of this package insert) to each well using a repeater pipette.
6. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 min**. at room temperature (23 ± 2°C).
7. Aspirate and wash each strip **5 times** with Washing Solution **(350 µL/per well**) using an automatic microplate washer.
8. Add **100 µL** of the Biotin Conjugate RTU Solution to each well using a repeater pipette.
9. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **15 min**. at room temperature (23 ± 2°C).
10. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.
11. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
12. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **15 minutes** at room temperature (23 ± 2°C).
13. Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well**) using an automatic microplate washer.
14. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
15. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **12 min** at room temperature (23 ± 2°C).
    ***NOTE**: Visually monitor the color development to optimize the incubation time.*
16. 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 Inhibin A 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. Optimum results can be obtained at incubation temperature of **23 ± 2°C**.
2. Calculate the mean OD for each Calibrator, Control, or Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the Inhibin A concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the Inhibin A concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Inhibin A concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (CAL A) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.

LIMITATIONS

The reagents supplied in this kit are optimized to measure Inhibin A levels in human 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 heterophile antibodies in the samples[5].

The OMQ Check ELISA results should be interpreted with respect to the total clinical presentation of the patient, including symptoms, clinical history, data from additional tests, and other appropriate patient examination information.

The OMQ Check ELISA is not validated for use in prenatal screening to detect Down Syndrome, preeclampsia, ovarian tumors/cancers, and prediction of pregnancy in women undergoing IVF or to determine gonadal maturity in children.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- OMQ Check ELISA assay controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Inhibin A 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	Well Contents	Mean OD	Conc (pg/mL)
A1, A2	**Calibrators** A	0.014 (Blank)	0
B1, B2	B	0.032	41.0
C1, C2	C	0.130	185.0
D1, D2	D	0.389	625.0
E1, E2	E	1.304	2378.0
F1, F2	F	2.802	6027.0

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

ANALYTICAL CHARACTERISTICS

Analytical Sensitivity:

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 pg/mL) and calibrator B (41 pg/mL) is 6.58 pg/mL.

Limit of Blank (LoB):

The Limit of Blank was 1.417 pg/mL, calculated from a minimum of n=18 measurements of analyte free sample.

Limit of Detection (LoD):

The lowest amount of Inhibin A in a sample that can be detected with a 95% probability (n=24) is 7.7 pg/mL. The value was determined by processing seven serum samples in the range of 11.4 to 89.8 pg/mL following CLSI EP17 guidelines.

Limit of Quantitation (LOQ):

The estimated minimum dose achieved at 20% total imprecision is 12.58 pg/mL. The value was determined by processing six samples in the range of 6.7-65.1 pg/mL over eight runs and two days in duplicates (n=24) following CLSI EP17 guidelines.

Imprecision:

Reproducibility of the OMQ Check assay was determined in a study using three serum pools. The study included a total of 6 assays, three replicates of each per assay (n=18). Controls included a total of 6 assays, two replicates of each per assay (n=12). Representative data were calculated based on CLSI EP5-A2 guidelines and are presented in the following table.

Sample	Mean Conc.	Within Run		Between Run		Total	
	(pg/mL)	SD	%CV	SD	%CV	SD	%CV
Control I	1256.099	16.194	1.29	81.483	6.49	83.077	6.61
Control II	3682.014	152.200	4.13	185.516	5.04	239.961	6.52
Pool-1	87.790	3.488	3.97	1.436	1.64	3.772	4.30
Pool-2	630.888	15.311	2.43	11.616	1.84	19.219	3.05
Pool-3	3393.514	103.530	3.05	92.662	2.73	138.941	4.09

Recovery:

Known amounts of Inhibin A were added to five serum samples containing different levels of endogenous Inhibin A. The concentration of Inhibin A was determined before and after the addition of exogenous Inhibin A and the percent recovery was calculated.

Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	172.0	464.7	463.5	100%
		757.5	751.6	99%
		1050.2	1083.4	103%
2	365.1	648.2	731.1	113%
		931.3	987.4	106%
		1214.4	1308.2	108%
3	307.0	593.0	528.8	89%
		879.0	777.7	88%
		1165.0	1060.2	91%
4	165.3	458.4	496.1	108%
		751.5	766.6	102%
		1044.6	1088.3	104%
5	261.1	549.4	599.4	109%
		837.7	942.4	113%
		1126.0	1275.5	113%

Linearity:

Based on CLSI EP6P-A multiple dilutions of the Calibrator F and two serum samples containing various Inhibin A levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	Individual %Recovery	% Recovery
Cal. F	Neat	6027.000			102%
	1:2	3013.500	3122.814	104%	
	1:4	1506.750	1542.379	102%	
	1:8	753.375	759.594	101%	
	1:16	376.688	380.957	101%	
	1:32	188.344	194.858	103%	
1	Neat	511.217			90%
	1:2	255.609	239.508	94%	
	1:4	127.804	114.229	89%	
	1:8	63.902	54.745	86%	
	1:16	31.951	28.560	89%	
2	Neat	435.876			92%
	1:2	217.938	198.408	91%	
	1:4	108.969	103.890	95%	
	1:8	54.485	48.111	88%	
	1:16	27.242	25.372	93%	

Analytical Specificity:

This monoclonal antibody pair used in the assay detects Inhibin A. Other related molecules at the concentration in the table below did not show any significant cross-reaction.

Sample	Cross-reactant	Concentration	% Cross-reactivity
1	Inhibin B	50 ng/mL	ND
2	H Activin A	50 ng/mL	ND
3	Activin B	50 ng/mL	ND
4	Activin AB	50 ng/mL	ND
5	Myostatin	50 ng/mL	ND
6	hAMH	50 ng/mL	ND
7	Follistatin 288	50 ng/mL	ND
8	Follistatin 315	50 ng/mL	ND
9	LH	50 ng/mL	ND
10	hFSH	50 ng/mL	ND
11	α-2-macroglobulin	50 ng/mL	ND
12	AFP	50 ng/mL	ND
13	PAPP-A	50 ng/mL	ND
14	hCG	50 ng/mL	ND

Species Immunoreactivity:

The antibody pair used in OMQ Check assay detects Bovine and Equine samples. The antibody pair does not detect Goat, Rabbit, Ovine, Porcine, Canine, or Mouse samples.

Expected Value:

Serum samples were analyzed using Ansh OMQ Check ELISA. The expected ranges for Inhibin A were calculated using 95% non-parametric estimation using Analyse-It® for Microsoft Excel

Population	No of specimens (n)	Median conc. (pg/mL)	OMQ Check (pg/mL)
Normal Cycling Females			
21 - 54	86	14.5	15.2 – 78.8
Gestational (Weeks, days)			
9 - 13	82	268.3	240.8 – 287.3
16,0 – 20,4	105	158.6	139.5 – 187.5
39 - 40	10	438.6	182.1 – 610.2

Note: *It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.*

Interference:

When Hemoglobin, Biotin, Intralipids, and Bilirubin were added at greater than two folds of their physiological concentration to control sample, Inhibin A concentrations were within ± 10% of the control as represented in the following table. This study was based on CLSI EP7-A2.

Interferents	Analyte Conc. (pg/mL)	Unspiked Sample Value (pg/mL)	Spiked Sample Value (pg/mL)	% Difference
Hemoglobin	1 mg/mL	355.75	354.96	-0.22
	0.5 mg/mL	365.26	362.08	-0.87
	0.1 mg/mL	352.59	359.71	2.02
Hemoglobin	1 mg/mL	929.14	913.57	-1.68
	0.5 mg/mL	921.38	897.18	-2.63
	0.1 mg/mL	872.23	894.60	2.56
Biotin	1200 ng/mL	357.34	372.39	4.21
	600 ng/mL	362.87	370.01	1.97

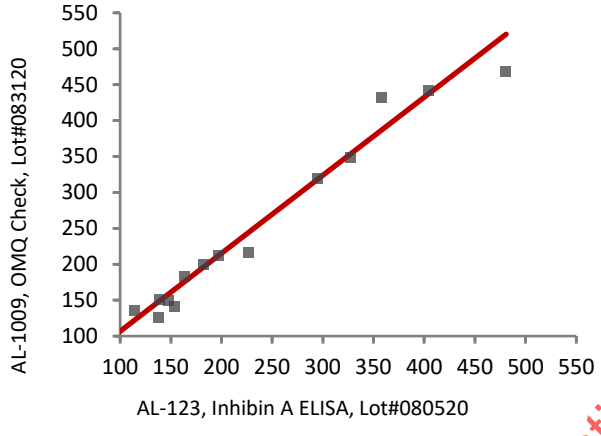
	200 ng/mL	351.81	377.18	7.21
Biotin	1200 ng/mL	901.50	900.63	-0.10
	600 ng/mL	884.27	887.73	0.39
	200 ng/mL	898.91	885.99	-1.44
Intralipids	20 mg/mL	311.66	337.59	8.32
	10 mg/mL	336.81	343.11	1.87
	5 mg/mL	332.86	346.28	4.03
Intralipids	20 mg/mL	792.79	847.37	6.88
	10 mg/mL	816.63	880.87	7.87
	5 mg/mL	861.14	878.26	1.99
Bilirubin	0.66 mg/mL	241.16	246.56	2.24
	0.2 mg/mL	321.66	331.17	2.96
Bilirubin	0.66 mg/mL	605.57	614.83	1.53
	0.2 mg/mL	857.69	847.24	-1.22

Method Comparison:

The OMQ Check ELISA (AL-1009) has been compared to Inhibin A ELISA (AL-123) using 15 samples.

Passing Bablok analysis of the results yielded the following Regression:

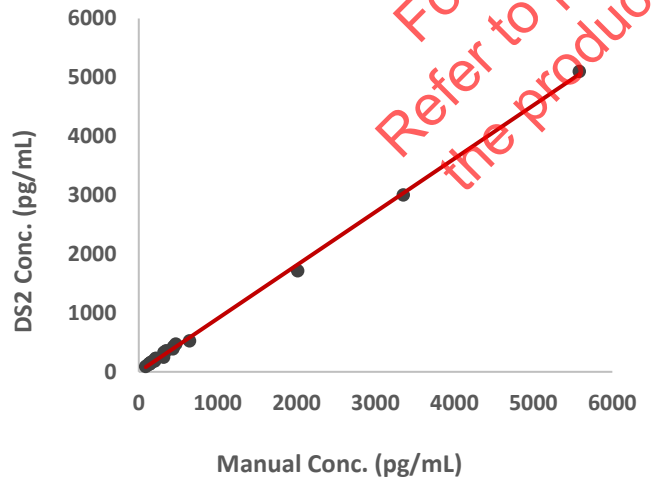
OMQ Check ELISA (AL-1009) = -1.931 + 1.086 Inhibin A (AL-123), r=0.985



Method Comparison: Manual vs. Automated

The OMQ Check ELISA (AL-1009) manual and automated platform (DS2 Dynex Immune Analyzer) have been compared using 15 samples.

Analysis of the results yielded the following: Slope: 90.54%, r=0.99



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This assay is intended for *in vitro* research use only.

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