picoAMH ELISA

AL-124-r

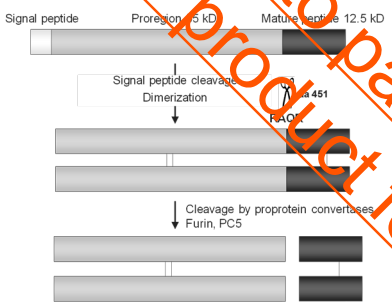
RUO

INTENDED USE

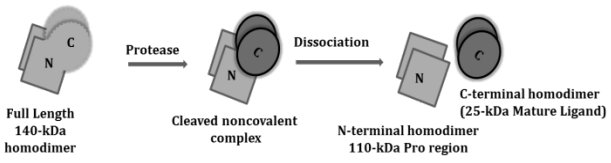
The picoAMH (Anti-Müllerian hormone) enzyme linked immunuosorbent assay (ELISA) kit provides materials for the quantitative measurement of ultra-low concentrations of AMH in human serum and other biological fluids. This assay is intended for research use only.

SUMMARY AND EXPLANATION

Anti-Müllerian hormone (AMH), a member of the TGFβ superfamily, is a homodimeric glycoprotein composed of two 55 kDa N-terminal and two 12.5 kDa C-terminal homodimers, non-covalently linked by disulfide bridges. Processing of AMH is shown below.[1]



Recent studies have shown that the AMH C-terminal homodimer is much less active than the noncovalent complex, but almost all activity can be restored by associating with the N-terminal pro-region, which reforms a complex with the mature C-terminal homodimer. This finding raises the possibility that the AMH noncovalent complex is the active form of protein. It was reported that the cleaved AMH noncovalent complex binds to AMHRII and stimulates intracellular signaling, whereas full-length AMH shows only minimal activity.[2]



AMH is secreted by the Sertoli cells in males. During embryonic development, AMH is responsible for Müllerian duct regression. AMH continues to be produced by the testes until puberty and then decreases slowly to residual post-puberty values. In females, AMH is produced by the granulosa cells of small growing follicles from the 36th week of gestation onwards until menopause when levels become undetectable. Potential clinical applications of low end anti-müllerian hormone (AMH) have been published in premature ovarian insufficiency,[3] ovarian tumors,[4-5] menopause[6-8] and many more.

PRINCIPLE OF THE TEST

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

MATERIALS SUPPLIED

CAL-124A picoAMH Calibrator A / Sample Diluent

One vial, 10 mL, labeled AMH Cal A/Sample Diluent, containing 0 pg/mL AMH in serum with non-mercury preservative. Store unopened at 2-8°C until the expiration date.

CAL-124B - CAL-124F picoAMH Calibrators B thru F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 10-1000 pg/mL AMH in serum with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use and discard after the run. Avoid repeated freeze thaws.

Assay Calibration: The recombinant AMH concentrations in calibrators are standardized to purified recombinant mature AMH preparation that is characterized by mass spectroscopy and optical density at 280 nm. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-124-I & CTR-124-II picoAMH Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high AMH concentrations in serum with non-mercury preservative. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use and discard after the run. Avoid repeated freeze thaws.

PLT-124 AMH/MIS Coated Microtitration strips

One stripholder, containing 12 strips and 96 microtitration wells with AMH 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-205 AMH/MIS Assay Buffer

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

BCR-124 picoAMH Biotin Conjugate Ready-To-Use (RTU)

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

SAR-124 picoAMH Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)

One amber 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 2-30°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. Repeator pipette
6. Vortex mixer.
7. Deionized water.

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

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.[9]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin 300 and Sodium azide[10] 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 and Lithium heparin plasma 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) Within two hours after centrifugation, transfer at least 500 µL of cell free sample to a storage tube, vortex and tightly stopper the tube immediately.

d) Samples may be stored at 4°C if assayed within 7 days; otherwise samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

e) Avoid assaying lipemic, hemolyzed or icteric samples.

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

g) 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.[11]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the picoAMH 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 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. **picoAMH Calibrators B-F and picoAMH Controls I & II**: Tap and reconstitute picoAMH Calibrator B-F and picoAMH Controls I & II each with 1 mL deionized water. Solubilize, mix well and use after reconstitution.
   Note: In case sensitivity below calibrator B level is desired, dilute reconstituted calibrator B as below.
2. (a) CAL B/2: Mix 150 µL of reconstituted Cal B with 150 µL of Cal A/Sample diluent.
   (b) CAL B/3: Mix 100 µL of reconstituted Cal B with 200 µL of Cal A/Sample diluent.
3. **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.
4. **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.

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.

NOTE: *All serum/plasma samples reading higher than the highest calibrator should be diluted as appropriate in the 0 pg/mL Calibrator A/Sample diluent prior to assay.*

In general, pediatric males (<3yrs) subject samples should be ***diluted 1-part*** *serum/plasma in* ***20 parts*** *of calibrator A (10µl of sample + 190µl of Cal-124A/Sample diluent) prior to assay in protocol-1*

Protocol-1 (Female ≤ 40yrs)

1. Label the microtitration strips to be used.
2. Add **50 µL** of the **AMH/MIS Assay Buffer** to each well using a repeater pipette.
3. Pipette **100 µL** of the reconstituted **Calibrator and Controls** to the appropriate wells.
4. Pipette **10 µL** of **samples** using precision pipette to the sample designated wells.
5. Pipette **90 µL of Cal-124A/Sample-diluent** to the sample added wells.
6. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **3 hrs** at room temperature (23 ± 2°C).
7. Aspirate and wash each strip **5 times** with Wash Solution **(350 µL/per well)** using an automatic microplate washer.
8. Add **100 µL** of the **Antibody-Biotin Conjugate RTU** 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 **1 hr** 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 **30 mins** 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 **8-12 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 **10 minutes**, using a microplate reader set to **(1) 450 nm, (2) 405 nm and 630 nm (machine blank).**
17. **IMPORTANT:** All diluted specimens should be multiplied by the appropriate dilution factor (**i.e. 10**) for the final concentration. Alternatively, multiply the calibrators by **a factor of 10** and input the corrected calibrator values in the data reduction software of the spectrophotometer prior to reading the assay.

NOTE*: a) While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a "****Blank****".*

b) Each lab should establish their extrapolation criteria 1) ***450 nm-630 nm*** *at the low end and* ***405 nm-630 nm*** *at the high end of the curve if needed.*

c) All diluted samples reading lower than the limit of detection should be run following protocol-2.

Protocol 2 (Female age > 40yrs) *
Females of all ages with diminished ovarian reserve

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.

NOTE: *All serum/plasma samples reading higher than the highest calibrator should be diluted as appropriate in the 0 pg/mL Calibrator A/Sample diluent prior to assay.*

1. Label the microtitration strips to be used.
2. Add **50 µL** of the **AMH/MIS Assay Buffer** to each well using a repeater pipette.
3. Pipette **100 µL** of the reconstituted **Calibrator and Controls** to the appropriate wells.
4. Pipette **50 µL of samples** using precision pipette to the sample designated wells.
5. Pipette **50 µL of Cal-124A/Sample-diluent** to the sample added wells.
6. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **3 hrs** at room temperature (23 ± 2°C).
7. Aspirate and wash each strip **5 times** with Wash Solution **(350 µL/per well)** using an automatic microplate washer.
8. Add **100 µL** of the **Antibody-Biotin Conjugate RTU** 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 **1 hr**. 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 **30 mins** 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 **8-12 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 10 minutes, using a microplate reader set to to (1) 450 nm, (2) 405 nm and 630nm (machine blank).
17. **IMPORTANT:** All diluted specimens should be multiplied by the appropriate dilution factor (**i.e. 2**) for the final concentration. Alternatively, multiply the calibrators by **a factor of 2** and input the corrected calibrator values in the data reduction software of the spectrophotometer prior to reading the assay.

NOTE*: a) While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a "****Blank****".*

b) Each lab should establish their extrapolation criteria 1) ***450 nm-630 nm*** *at the low end and* ***405 nm-630 nm*** *at the high end of the curve if needed.*

c) All diluted samples reading lower than the limit of detection can be run neat (100 ul sample in step-4 and skip step-5) in protocol-2. No calibration factor is required if run neat.

Combination Protocol

NOTE: Protocol-1 and Protocol-2 can also be performed simultaneously in the same run by marking the sample wells as per the protocol used. The sample results then should be processed as per the protocol by applying the protocol calibration factor.

RESULTS

NOTE: *The results in this package insert were calculated by plotting the* ***log optical density (OD) data on the y-axis and log AMH concentration on X-axis*** *using a cubic regression curve-fit. 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 optical density (OD) for each calibrator, Control, or unknown samples.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the AMH concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the AMH concentrations of the Controls and unknown samples from the calibration curve by matching their mean OD readings with the corresponding AMH concentrations.
5. All serum/plasma samples reading higher than the highest calibrator should be diluted as appropriate in the 0 pg/mL Calibrator A/Sample diluent and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the diluted sample value by the dilution factor, if required.

LIMITATIONS

The reagents supplied in this kit are optimized to measure AMH levels in human serum and lithium heparin plasma. 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.[12]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- picoAMH ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for picoAMH 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 Absorbance	Conc (pg/mL)	Conc (pMol/L)
A1, A2	Calibrator A	0.022 (**Blank**)	0	0
B1, B2	B/2	0.015	3.8	0.027
C1, C2	B	0.03	7.6	0.054
D1, D2	C	0.111	31	0.221
E1, E2	D	0.375	104.7	0.748
F1, F2	E	1.148	360.2	2.572
G1, G2	F	2.917	1091	7.789

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

The performance characteristic results are reported in pg/mL and can be converted to pmol/L using the conversion factor below.

1pg/mL = 0.00714pMol/L

Analytical Sensitivity

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 90 replicates of calibrator A (0 pg/mL) and calibrator B (6.0 pg/mL) is 1.96 pg/mL or 0.014 pMol/L.

Limit of Detection (LoD):

The lowest amount of AMH in a sample that can be detected with a 95% probability (n=90) is 1.2 pg/mL or 0.0086 pMol/L. The value was determined by processing four serum samples in the range of 1.7 pg/mL to 11.6 pg/mL following CLSI EP17 guidelines. Nine assay runs per day were performed over five days with all samples run in duplicate per run.

Limit of Quantitation (LoQ):

The estimated minimum dose achieved at 20% total imprecision is 3.9 pg/mL or 0.028 pMol/L. The value was determined by processing seven samples in the range of 1.7 -371 pg/mL over forty-five runs and five days in duplicates (n=90) following CLSI EP17 guidelines.

Imprecision:

Reproducibility of the picoAMH ELISA assay was determined in a study using two kit controls and three serum pools. The study included a total of 40 assays, two replicates of each per assay (n=80). Representative data were calculated based on CLSI EP5-A guidelines and are presented in the following table.

Sample	Conc. (pg/mL)	Within run		Between run		Total	
		SD	CV	SD	CV	SD	CV
Control I	64.063	1.837	2.87%	2.039	3.18%	2.745	4.28%
Control II	186.440	6.520	3.50%	5.810	3.12%	8.733	4.68%
QC1	22.575	0.834	3.69%	1.020	4.52%	1.317	5.84%
QC2	86.509	1.899	2.20%	1.950	2.25%	2.722	3.15%
QC3	373.181	7.729	2.07%	14.230	3.81%	16.194	4.34%

Recovery

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

Sample ID	Endogenous value in pg/mL	Expected in pg/mL	Observed in pg/mL	%Recovery
1	121.152	150.907	154.492	102%
		177.956	174.916	98%
		202.654	201.542	99%
2	130.113	159.441	159.102	100%
		186.103	182.422	98%
		210.446	218.860	104%
3	113.449	143.570	138.990	97%
		170.954	169.920	99%
		195.956	199.061	102%
4	218.857	243.769	257.295	106%
		266.197	265.247	99%
		287.141	286.793	100%

Linearity:

Based on CLSI EP-6-P multiple dilutions of the three serum samples and calibrator F (recombinant AMH antigen) containing various AMH levels were diluted with Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	Neat	449.171	NA	NA
	1:2	224.586	211.758	94%
	1:4	112.293	105.736	94%
	1:8	56.146	55.442	99%
	1:16	28.073	27.095	97%
2	1:10	558.693	Neat	NA
	1:20	279.347	298.668	107%
	1:40	139.673	142.487	102%

	1:80	69.837	66.977	96%
	1:160	34.918	33.074	95%
3	1:20	619.344	Neat	NA
	1:40	309.672	318.049	103%
	1:80	154.836	166.197	107%
	1:160	77.418	79.615	103%
	1:320	38.709	37.350	96%
Calibrator F Human AMH Antigen	Neat	746.0	NA	NA
	1:2	373.0	377.19	101%
	1:4	186.5	179.874	96%
	1:8	93.25	87.984	94%
	1:16	46.625	44.717	96%
	1:32	23.313	23.054	99%

Analytical Specificity:

This monoclonal antibody pair used in the assay is specific for human AMH and does not detect rat, mouse, porcine, equine, bovine, canine and ovine AMH. The antibody pair chosen does not cross reactivity to Inhibin A, Inhibin B, Activin A, Activin B and Activin AB.

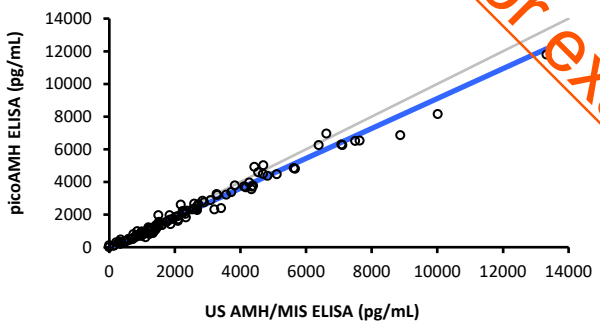
Interference:

When potential interferents (hemoglobin, triglycerides and bilirubin) were added at least at two times their physiological concentration to control sample, AMH concentration were within ± 10% of the control.

Method Comparison:

The picoAMH ELISA has been compared to Ultra-Sensitive AMH/MIS ELISA (AL-105) using 115 serum samples in the range of 0 - 13300 pg/mL. Passing & Bablok fit analysis of the results yielded the following Regression:

picoAMH ELISA (AL-124) = 0.92 US AMH/MIS ELISA (AL-105) – 50.69
(rs=0.99 P<0.0001)



REFERENCES

1. Pepinski, R.B., et al. (1988) J. Biol. Chem., 263, 18961-18964.
2. Di Clemente et al. Mol Endocrinol, November 2010, 24 (11): 2193-2206.
3. Méduri G, et. Al.Hum Reprod. 2007;22: 117-23.
4. Lee M, Donahoe et al. Journal of Clinical Endocrinology and Metabolism 1996; 81:571-575.
5. Anderson R.A et al. Human Reproduction 2006, Vol.21 (10): 2583–2592.
6. Robertson, D.M et al. Menopause 2014 Dec; 21(12):1277-1286.
7. Freeman EW, et al. 2012. J Clin Endocrinol Metab;97:1673-1680.
8. Tehrani FR, et al. J Clin Endocrinol Metab 2013; 98:729–735.
9. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
10. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http:// www.cdc.gov/niosh.
11. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
12. 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.
Webster, TX 77598-4217, U.S.A.

PicoAMH ELISA PROTOCOLS

Steps	Procedure	Protocol-1 (Female ≤ 40yrs)	Protocol-2 (Female > 40yrs)* **Females* **of all ages** ***with diminished ovarian reserve***
1.	a.	Label Microtitration strips to be used.	
	b.	Add **50µL** of **AMH/MIS Assay Buffer** to each well.	
	c.	Add **100 µL** of the reconstituted **Calibrator, Controls** to the designated wells.	
	d.	Add **10 µL** of the **samples** to the designated wells.	Add **50 µL** of the **samples** to the designated wells.
	e.	Add **90 µL** of **Cal-124A/Sample-diluent** to the sample added wells.	Add **50 µL** of the **Cal-124A/Sample-diluent to** the sample added wells.
	f.	Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **3 hrs** at room temperature (23 ± 2°C).	
2.		Aspirate and wash each strip **5 times** with Wash Solution **(350 µL/per well)** using an automatic microplate washer.	
3.	a.	Add **100 µL** of the **Antibody Biotin Conjugate RTU** to each well using a repeater pipette.	
	b.	Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **1 hr** at room temperature.	
4.		Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.	
5.	a.	Add **100 µL** of the **Streptavidin-Enzyme Conjugate-RTU** to each well using a repeater pipette.	
	b.	Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature.	
6.		Aspirate and wash each strip **5 times** with the Wash Solution **(350 µL/per well)** using an automatic microplate washer.	
7.	a.	Add **100 µL** of the **TMB chromogen solution** to each well using a repeater pipette. Avoid exposure to direct sunlight.	
	b.	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.*	
8.	a.	Add **100 µL** of the **Stopping solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **10 minutes**, using a microplate reader set to (1) **450 nm, (2) 405 nm and 630nm (machine blank).**	
9.	a.	Multiply the calibrators by **a factor of 10** prior to data reduction.	Multiply the calibrators by **a factor of 2** prior to data reduction.

NOTE:

Protocol-1 and Protocol-2 can also be performed simultaneously in the same run by marking the sample wells as per the protocol used. The sample results then should be processed as per the protocol by applying the protocol calibration factor.