

PERFORMANCE CHARACTERISTICS

A) Precision data

Two concentration levels of AMH samples were used to determine the inter precision of AMH ELISA assay kit.

Concentrations	No. of testing	Range	SD	CV
Level 1	10	0.1-0.6	0.153	4.7%
Level 2	10	5-15	0.862	5.9%

B) Internal Evaluation:

In an internal Study **Qualisa™ AMH** was evaluated against commercially available licensed kit with 90 random clinical samples and **Qualisa™ AMH** has demonstrated 100% clinical correlation with the commercially available licensed kit.

C) External Evaluation:

Qualisa™ AMH ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ AMH** ELISA has demonstrated 100% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics (P) Ltd).

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.












LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

(1) Hampf, R.; Snajderova, M.; Mardetal, T. (2011)., Antimullerian hormone (AMH) not only a marker for prediction of ovarian reserve". *Physiological research/Academia Scientiarum Bohemoslovaca* 60 (2): 217-223. (2) Visser J. de Jong F, Laven J, Themmen A (2006). Anti-Mullerian hormone: a new marker for ovarian function". *Reproduction* 131(1): 1-9. (3) Approved Guideline-Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute. (4) Bjerner J, Nustad K, Norum LF, Olsen KH, Bormer OP. 2002. Immunometric assay interference: incidence and prevention. *Clin Chem* 48:613-621. (5) Lee, M. et al (1993); Mullerian-inhibiting substance: A gonadal hormone with multiple functions. *Endocrine Reviews*, 14, 152-164. (6) Hudson, et al (1990); An immunoassay to detect human Mullerian Inhibition substance in males and females during normal development. *Journal of Clinical Endocrinology and Metabolism*, 70, 16-22.

SYMBOL KEYS

 Temperature Limitation	 Consult Instructions for use	 Date of Manufacture	 Batch Number / Lot Number
 Manufacturer	 In vitro Diagnostic Medical Device	 This side up	 Contains sufficient for <n> tests
 Use by	 Catalogue Number	 Do not reuse	



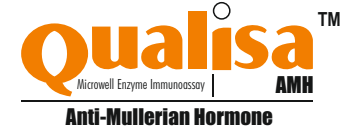
Manufactured by:

Zephyr Biomedicals

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Enzyme Immunoassay for the Quantitative Determination of Anti-Mullerian Hormone (AMH) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ AMH test is intended for the quantitative determination of Anti-Mullerian Hormone (AMH) levels in human serum and plasma. For In Vitro Diagnostic Use Only.

INTRODUCTION

Anti-Mullerian hormone is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis. AMH expression is critical to sex differentiation at a specific time during fetal development and appears to be tightly regulated by nuclear receptor SF1, transcription GATA factors, sex-reversal gene DAX1, and follicle-stimulating hormone (FSH). AMH is activated by SOX9 in the Sertoli cells of the male fetus thereby arresting the development of fallopian tubes, uterus, and upper vagina. AMH is also a product of granulosa cells of the preantral and small antral follicles in women. As such, AMH is only present in the ovary until menopause. AMH level is also lower and even below the detection limit if women with premature ovarian failure of any cause, including after cancer chemotherapy, etc.

PRINCIPLE

The **Qualisa™ AMH** Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. In this assay, standards and samples are directly incubated in micro titer wells which have been coated with anti-AMH antibody. After the first incubation and washing, another anti-AMH detection antibody labeled with horseradish peroxidase (HRP) is added to each well. After a second incubation and washing step, a "sandwich" of solid-phase antibody-human AMH-HRP-conjugated monoclonal antibody is formed. Following the substrate tetramethylbenzidine (TMB) is added to the wells. The reaction is stopped after specified time using stop solution and the absorbance is determined for each well using an ELISA reader. The enzymatic activity of the immunocomplex in each well is directly proportional to the amount of human AMH in the test samples. The AMH concentrations in the samples can then be calculated from this standard curve.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti-AMH –antibody
- AMH Sample Diluent. Ready to use.
- AMH Enzyme Conjugate (100X)
- AMH Conjugate Diluent
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- AMH Standard set of 6 standards labeled as A to F in Lyophilized form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided:

- Precision pipettes: 10µl, 50-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ AMH** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to white at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to Standard procedure.
2. Only serum should be used.

3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

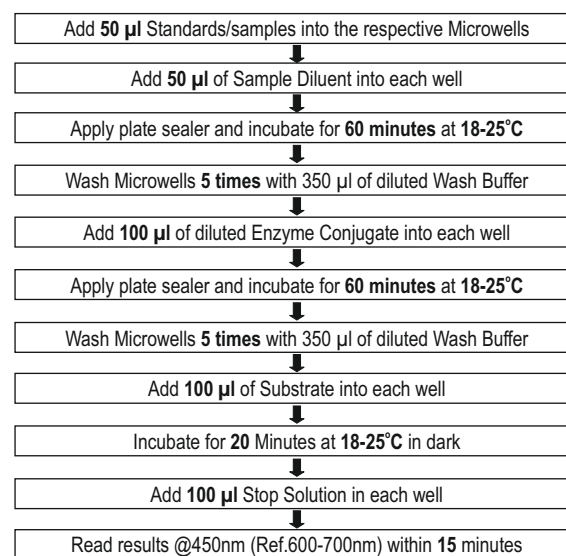
REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
3. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.
4. Since the reference standards are lyophilized, reconstitute each standard with 1 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Enzyme Conjugate (µl)	10	20	30	35	45	50	60	70	75	85	90	100
Conjugate Diluent (µl)	1000	2000	3000	3500	4500	5000	6000	7000	7500	8500	9000	10000

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50 µl** of standards and serums into the appropriate wells.
2. Dispense **50 µl** of Sample Diluent into each well. Incubate at room temperature (18-25°C) for **60 mins**.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense **100 µl** of diluted Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **60 mins**.
5. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Dispense **100 µl** of TMB Substrate into each well. Incubate at room temperature (18-25°C), in the dark, for **20 minutes**.
7. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
8. Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.



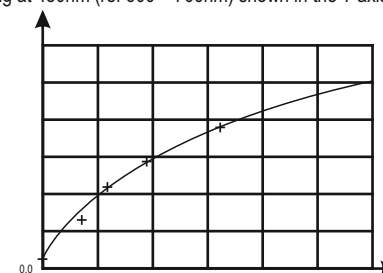
CALCULATION OF RESULTS

Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of AMH in ng/ml from the standard curve.

Example of Stanadard curve

Results of a typical standard run with optical density reading at 450nm (ref 600 – 700nm) shown in the Y axis against AMH concentrations shown in the X axis.

AMH (ng/ml)	Absorbance (450nm)
A	0.036
B	0.081
C	0.202
D	0.711
E	1.678
F	2.339



This Standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own Standards curve and data.

Expected Ranges of values

We tested 33 male samples, aged from 19 to 69 years old. All the values of these male samples were within the range of 0.7 to 19 ng/ml. Total of 34 female samples, aged from 13 to 61 years old, were also tested. The AMH value of the 29 female samples aged from 13-45 were within the range of 0.9-9.5 ng/ml; while the value of the other 5 female samples aged above 45 were <0.1 ng/ml. **Each laboratory should establish its own normal range based on gender and age.**

Gender	Age	Range (ng/ml)
Males	< 24 months	14~466
	24months~12 years	7.4~243
	> 12 years	0.7~19
Females	< 24 months	<4.7
	24months~12 years	<8.8
	13~45 years	0.9~9.5
	> 45 years	<0.1

The minimum detectable concentration of this assay is 0.073 ng/ml.