Anti-Müllerian hormone (AMH)

Anti-Mullerian hormone (AMH) is a dimeric glycoprotein of the TGF-β protein family, and it is produced by granulosa cells and Sertoli cells in women and men respectively. AMH is a gonadal hormone that regulates the primordial follicle transition rate and follicle-stimulating hormone (FSH) sensitivity in the smaller antral follicles in females (1). AMH is secreted by granulosa cells of small antral and preantral follicles, and it reflects the size of the pool of these follicles in women (2). AMH levels are stable during the menstrual cycle (3).

In females, AMH secretion by ovarian granulosa cells starts at approximately the 36 week point of gestation, reaching a peak at approximately 25 years of age. It then gradually declines until menopause, at which point it becomes undetected in the bloodstream. However, in males, AMH levels are high after birth and they remain so in the pre-pubertal period, before lower and stable AMH levels occur in adult life (4).

AMH as a biomarker in diagnostics

AMH is currently considered to be among the most reliable biomarkers for the assessment of ovarian reserve in women (5). The measurement of AMH can be used in fertility investigations in order to help to predict the response of women to ovarian stimulation, as well as to estimate the time to menopause and also to diagnose and monitor women with polycystic ovary syndrome (6).

Biochemistry of AMH

AMH has two forms circulating in the blood. The first is proAMH homodimer, which does not bind to AMH receptors. The second is the cleaved, AMH type 2 receptor–binding form, known as AMHN,C. The 140-kDa proAMH homodimer can be cleaved by proteases, yielding a 25-kDa C-terminal dimer (AMHC) and a 120-kDa N-terminal dimer(AMHN), which remain associated as a noncovalent complex (AMHN,C) (7) (see Figure 1 on page 2). The majority of modern day immunoassays are able to detect both forms of AMH in the human blood (8).

Reagents for assay development

Hytest provides several well-characterized murine monoclonal antibodies (MAbs) that are specific to human AMH. The antibodies were developed against a recombinant human AMH. In addition, we provide a recombinant AMH protein that could be used as a calibrator or a standard in assay development.

CLINICAL UTILITY

- **Assessment of ovarian reserve**
- **Prognosis of the response to controlled ovarian stimulation**
- **Assessment of menopausal status**

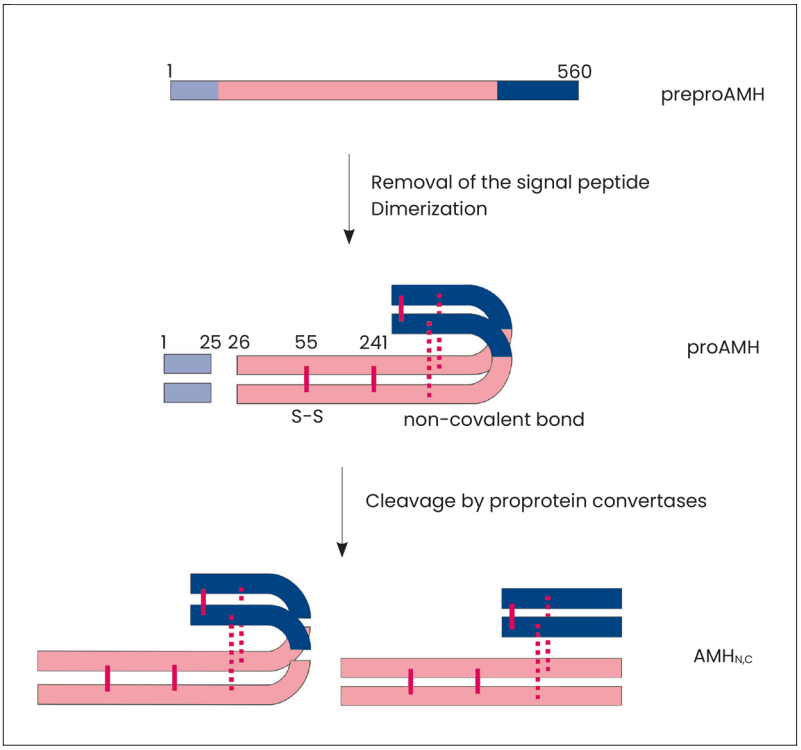

Figure 1.
Schematic representation of the formation of circulating AMH forms. Adapted from (9).

MONOCLONAL ANTIBODIES SPECIFIC TO AMH

All of our anti-AMH MAbs recognize the N-terminal part of AMH. Therefore, in sandwich immunoassays both forms of AMH will be detected. For an immunoassay development, we recommend four different antibody combinations that all provide high sensitivity (see Table 1). Calibration curves for recombinant AMH using two prototype assays in sandwich chemiluminescense immunoassay (CLIA) are provided in Figure 2.

Table 1.
Recommended pair combinations for sandwich immunoassays. Data is based on the results that were obtained with CLIA using either strepavidin/HRP or alkaline phosphatase for labeling. LoD= limit of detection.

Capture MAb	Detection MAb	LoD (pg/ml)
AMH65cc	AMH47cc	5
AMH69cc	AMH41cc	2
AMH69cc	AMH46cc	3
AMH60cc	AMH69cc	5

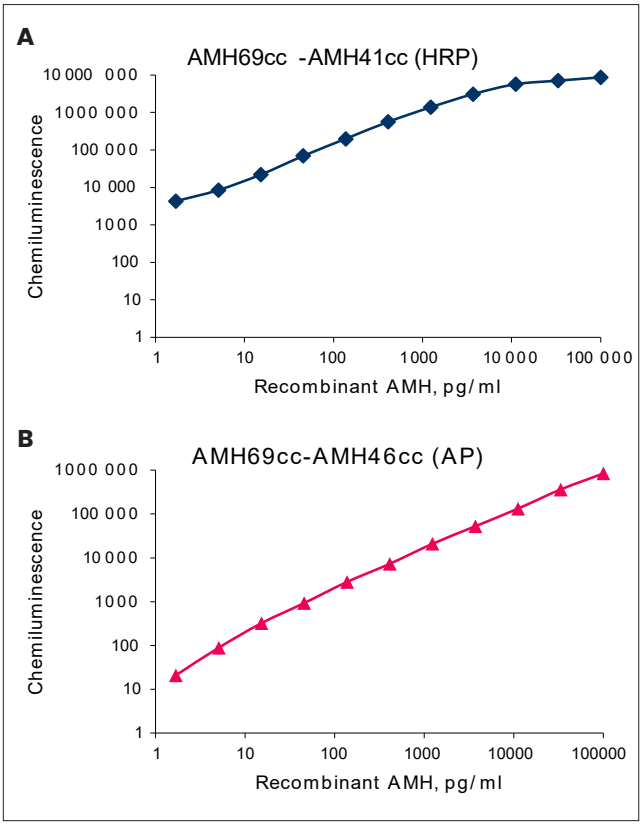

Figure 2.
Calibration curves for recombinant AMH in sandwich CLIA using AMH69cc-AMH41cc (A) and AMH69cc-AMH46cc (B). In (A) the label used was streptavidin/HRP. In (B) the detection antibody was labeled with alkaline phosphatase. Recombinant antigen was diluted with PBS containing 0.1% Tween 20 and 75 mg/ml BSA.

Detection of AMH in clinical samples

Measuring native AMH. The prototype assays can be used for the measurement of native AMH concentration in serum samples. In Figure 3, a dilution curve of native serum AMH was measured using AMH69cc-AMH46cc in CLIA. The sensitivity of the assay was approximately 5 pg/ml. The concentration of AMH was initially measured using the Beckman GEN II assay.

Prototype assays demonstrate a good correlation with commercial assays. Firstly, AMH was measured in serum samples that were obtained from 37 human beings aged between 2 – 46 years (samples from both genders were included) using the AMH69cc-AMH46cc and Beckman GEN II assays. The Beckman assay was performed according to the manufacturer's instructions. For the Hytest prototype assay, PBS containing 0.1% Tween 20 and 75 mg/ml BSA was used as the reaction buffer. Figure 4 shows that the two assays displayed good correlation with each other (R^2=0.93).

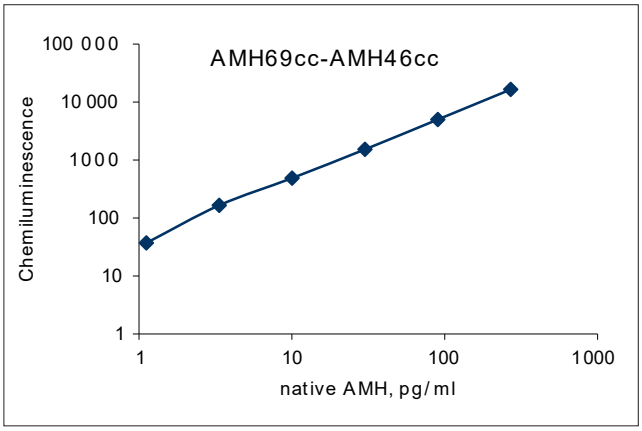

Figure 3.
Sandwich CLIA of native AMH in serum with alkaline phosphatase using the AMH69cc-AMH46cc prototype assay. The concentration of native serum AMH was initially determined by using the Beckman GEN II assay. A dilution series of serum was made in PBS containing 0.1% Tween 20, 75 mg/ml BSA.

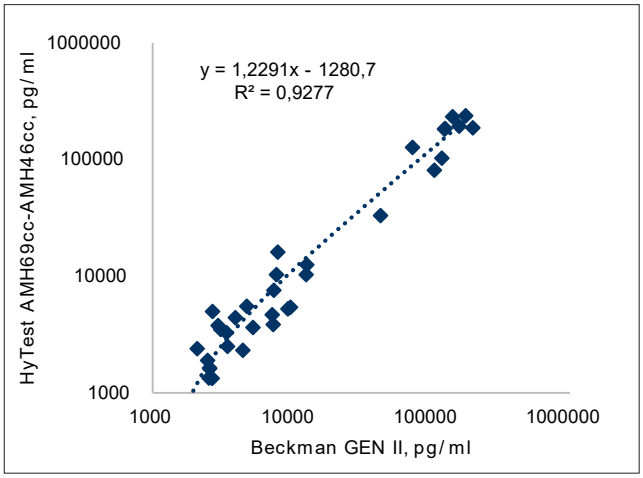

Figure 4.
AMH measurement results with the Hytest prototype assay AMH69cc-AMH46cc showed a good correlation with the Beckman GEN II assay.

Another comparison test was performed between the AMH69cc-AMH41cc and Roche Elecsys® AMH assays. Measuring 40 values ranging from 0 to 20 ng/ml showed a good correlation between these assays (see Figure 5).

RECOMBINANT HUMAN AMH

Hytest's recombinant AMH antigen can be used as a standard or a calibrator in immunoassays. The protein is expressed in a mammalian cell line and it does not contain any tags.

Dilutional linearity of recombinant and native AMH

In order to compare the immunoreactivity of recombinant and native AMH, a dilutional linearity study in which recombinant AMH was titrated along with a human serum sample was conducted. Calibration curves of both proteins were parallel, which indicates that the immunoreactivities behave similarly upon dilution (see Figure 6).

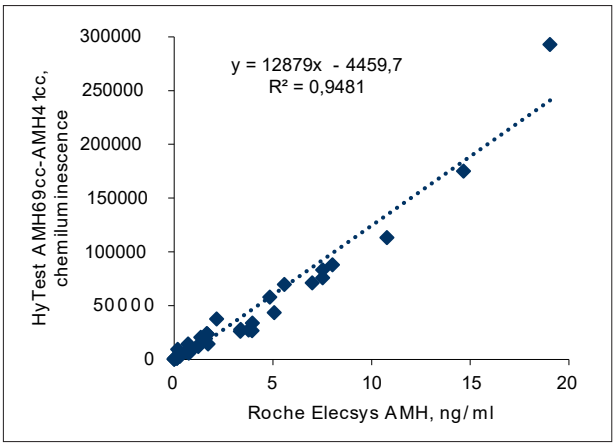

Figure 5.
AMH measurement results with the Hytest prototype assay AMH69cc-AMH41cc showed a good correlation with the Roche Elecsys AMH assay.

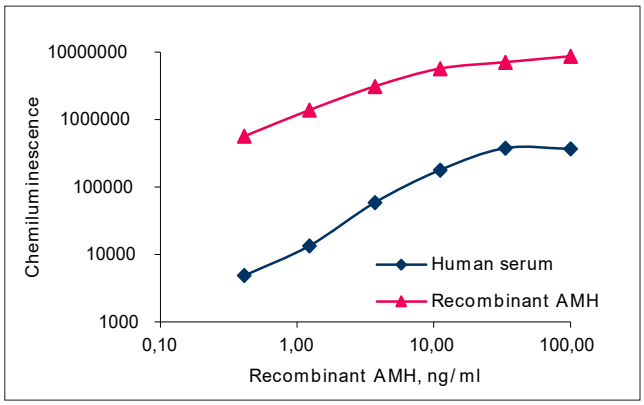

Figure 6.
Dilutional linearity for the MAb pair AMH69cc-AMH41cc. Serum was titrated alongside with the recombinant AMH in PBS containing 0.1% Tween 20 and 75 mg/ml BSA.

Recovery of recombinant AMH in serum

In order study the behavior of the recombinant AMH spiked into human serum, a recovery study was conducted (see Figure 7). At 100%, the signal from serum spiked with recombinant AMH would equal the sum of recombinant AMH in buffer and the endogenous AMH in serum. A good recovery (80-105%) was discovered over a wide range of recombinant AMH concentrations that were tested.

Batch-to-batch variations in immunochemical properties

AMH is a glycosylated homodimer with several cleavage sites and it is a target of various proteases. The ability of AMH and its proteolytic fragments to form covalent and non-covalent bonds modulates its immunochemical properties. The recombinant AMH undergoes proteolysis and the purified product contains fragments that are visible in SDS-PAGE (see Figure 8). The formation of fragments is the result of a biological process that may occur differently each time that recombinant AMH is expressed in a cell culture. Please note that this leads to batch-to-batch variation of the immunochemical properties of the product.

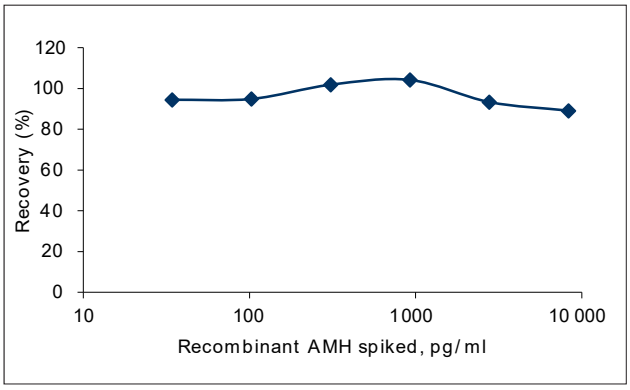

Figure 7.
Recovery of recombinant AMH spiked into human serum measured using the AMH69cc-AMH41cc prototype assay in CLIA.

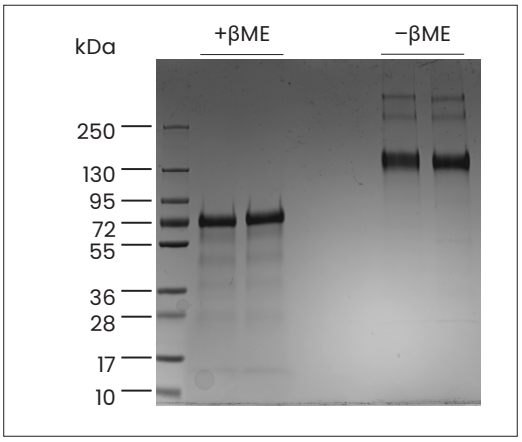

Figure 8.
SDS-PAGE of purified recombinant AMH (two batches) in reducing (+beta-mercaptoethanol, βME) and non-reducing conditions (-βME).
6 µg of protein was loaded per lane.

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Anti-Müllerian hormone (AMH), human	4AM5	AHM41cc	IgG2a	*In vitro*, EIA, WB
		AMH46cc	IgG2a	*In vitro*, EIA, WB
		AMH47cc	IgG2a	*In vitro*, EIA, WB
		AMH60cc	IgG2b	*In vitro*, EIA, WB
		AMH65cc	IgG1	*In vitro*, EIA, WB
		AMH69cc	IgG2b	*In vitro*, EIA, WB

ANTIGENS

Product name	Cat. #	Purity	Source
Anti-Müllerian hormone (AMH), human, recombinant	8AM7	>90%	Recombinant

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • **HYTEST.FI**