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.