

Biotech Trading Partners

QnE Hyaluronic Acid ELISA

(Singlet Testing)

Prepare working dilution of wash buffer by diluting one bottle of Wash Concentrate with 1L of distilled water.

1

Label the tubes and add 150µl of Reaction Buffer (Blue Solution) to a test tube for each of the reference solutions, controls and specimens to be tested. Add 15µl of each reference solution, control, and specimen to the specifically labeled tube. Mix well.

Open the Microplate pouch and remove the frame and number of strips needed for today's run. Replace the remaining strips into the pouch and reseal.

2

Water-Blank (EMPTY)		Medium Control
Reagent Blank (0ng./mL)		Low Control
50ng/mL Standard		Specimen #1
100ng/mL Standard		Specimen #2
200ng/mL Standard		Specimen #3
500ng/mL Standard		Specimen #4
800ng/mL Standard		Specimen #5
High Control		Specimen #6

Add the 100µl of the diluted reference solutions, controls, and specimens to the appropriate wells. The reagent blank gets 100µl of the reaction buffer only. The water-blank well is left empty.

Incubate for 60 Minutes at RT

WASH As Per Package Insert; the Water-Blank well can included in the wash cycle.

3

Water-Blank (EMPTY)		Medium Control
Reagent Blank (0ng./mL)		Low Control
50ng/mL Standard		Specimen #1
100ng/mL Standard		Specimen #2
200ng/mL Standard		Specimen #3
500ng/mL Standard		Specimen #4
800ng/mL Standard		Specimen #5
High Control		Specimen #6

Add the 100µl of the Conjugate Solution (red solution) to all wells **EXCEPT** the water-blank well. The water-blank well is left empty.

Incubate for 30 Minutes at RT

WASH As Per Package Insert; the Water-Blank well can included in the wash cycle.

4

Water-Blank (EMPTY)		Medium Control
Reagent Blank (0ng./mL)		Low Control
50ng/mL Standard		Specimen #1
100ng/mL Standard		Specimen #2
200ng/mL Standard		Specimen #3
500ng/mL Standard		Specimen #4
800ng/mL Standard		Specimen #5
High Control		Specimen #6

Add the 100µl of the Substrate Solution (brown bottle) to all wells **EXCEPT** the water-blank well. The water-blank well is left empty.

Incubate for 30 Minutes at RT.

Wells that have a positive reaction will develop a blue color.

















Do not WASH at the completion of this incubation.

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5

Water-Blank (see **)			Medium Control	Add the 100µl of the Stopping Solution (red capped bottle) to all wells <u>EXCEPT</u> the water-blank well.
Reagent Blank (0ng./mL)			Low Control	
50ng/mL Standard			Specimen #1	**At this time, add 200ul of reagent grade water to the Water-Blank well.
100ng/mL Standard			Specimen #2	
200ng/mL Standard			Specimen #3	Read the plate within an hour of adding Stopping Solution: 1) Read at 450nm with a reference of 650nm if available 2) Blank or zero the reader against the water-blank well.
500ng/mL Standard			Specimen #4	
800ng/mL Standard			Specimen #5	Plot ODs as per package insert to calculate the concentration of the specimens.
High Control			Specimen #6	