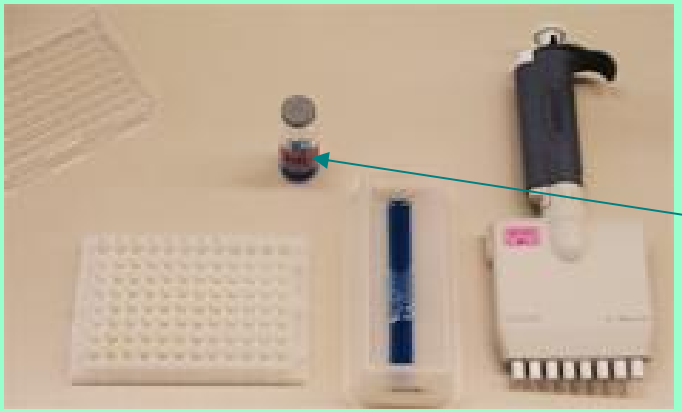
(1)Add 50 uL each of the diluted L-FABP Standard and sample to the **Pretreatment Microplate**.



(2)Further add 50 uL of the **Pretreatment Solution*** to each well using a continuous injection pipette.

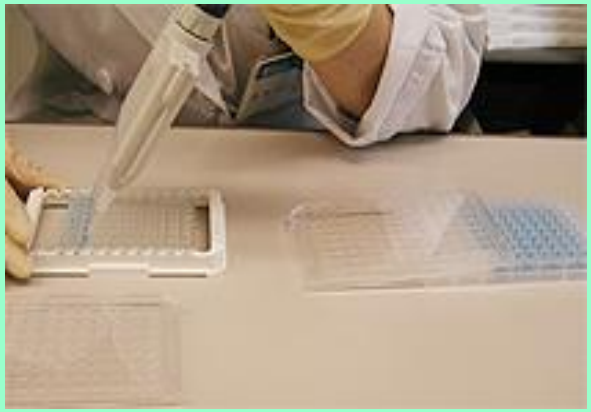
The **Pretreatment Solution should be brought to room temperature approximately 30 minutes before use.*

Pretreatment Solution

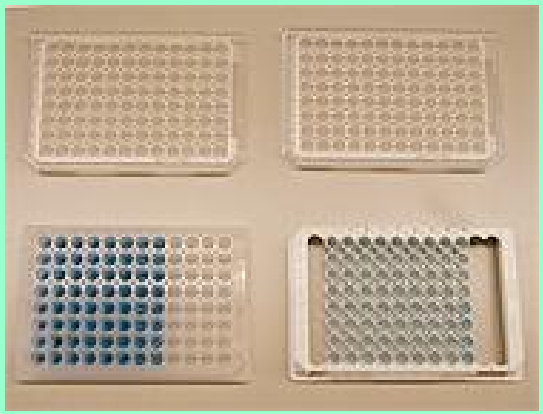
(3)Cover the plate with a plate seal and stir for 5 minutes with a plate mixer.

(4)Add 100 uL of the **Assay Buffer** to each well of the **L-FABP Antibody Coated Microplate**.

Assay Buffer



(5)Transfer 20 uL of the pretreated standard from the **Pretreatment Microplate** to the **L-FABP Antibody Coated Microplate** using a multichannel micropipette.



(6)Transfer 20 uL of the pretreated samples from the **Pretreatment Microplate** to the **L-FABP Antibody Coated Microplate** using a multichannel micropipette.

(7)Cover the L-FABP Antibody Coated Microplate with a plate seal and stir for 5 minutes with a plate mixer.

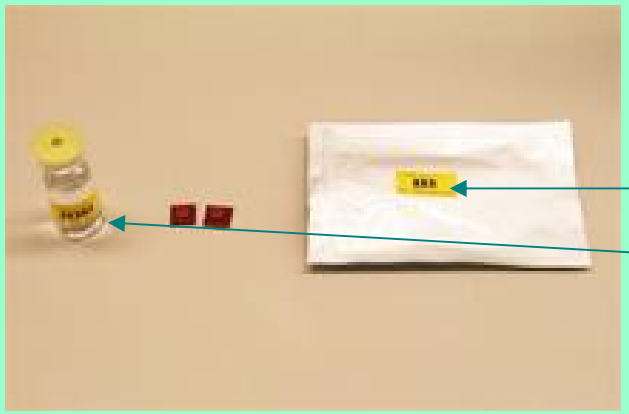
(8)Incubate 55 minutes at room temperature (20 to 28℃). Remove the reaction solution after completion of the incubation.

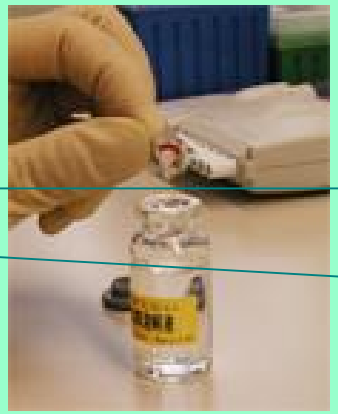
(9)Add 350 uL of the wash solution to each well and remove the wash solution. Repeat 3 times this washing procedure. (Even when a plate washer is used, wash 3 times with 350 uL of the wash solution.)

(10)Add 100 uL of **The 2nd Ab-POD Conjugate** to each well.

The 2nd Ab-POD Conjugate

(11)Cover the plate with a plate seal and stir for 5 minutes with a plate mixer. Incubate 55 minutes at room temperature (20 to 28℃).





(12)15minutes before end of reaction, make substrate solution.

NOTICE: Don’t tight seal with cap, because the substrate solution will be sparkling.

Substrate

Substrate Diluent



(13)Remove the reaction solution from the wells after completion of the incubation. Repeat washing following STEP(9) procedure.


(14) Add 100 uL of the substrate solution to each well.


(15) Cover the plate with a plate seal and stir for 5 minutes with a plate mixer. Incubate 25 minutes at room temperature(20 to 28℃) under light shielding.

(16) Add 100 uL of the **Stop Solution** to each well, and terminate enzyme reaction.

(17) Read the absorbance of each well at 492 nm using a microplate reader. If a dual wavelength plate reader is available, set the test wave length at 492 nm and reference at 630 nm.

Prepare a calibration curve based on the standard absorbance, and determine the L-FABP quantity in the samples.