ViroGates

Easy-to-use Guide for suPARnostic® AUTO Flex ELISA

CE IVD

2. Edition 2014

Preparation of buffers prior to use:

1. **Wash Buffer, working solution**
   Dilute the required amount of stock solution one plus nine parts (1:10) with distilled or deionized water. For the entire plate, the whole bottle should be added to 900 mL distilled or deionized water. For each strip to be used a minimum of 10 mL Wash Buffer should be prepared. More washing solution will be required, if an automatic plate washer is used. The working solution may be stored at 2 – 8˚C for up to 6 months.

2. **Peroxidase Conjugate, working solution**
   Prepare the required amount of Conjugate in a clean plastic container of suitable size as described in the table below (using Dilution Buffer, Component 6). Unused Conjugate stock should be protected from light and returned to 4˚C storage as soon as possible.

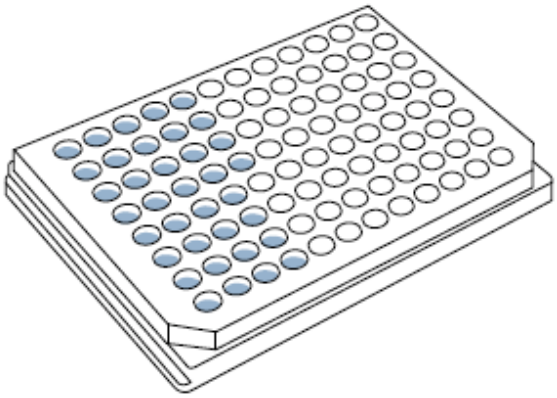
Number of strips	Volume of Conjugate stock (µL)	Volume of Dilution Buffer (mL)
1	15	3
2	20	4
3	25	5
4	30	6
5	35	7
6	40	8
7	45	9
8	50	10
9	55	11
10	60	12
11	65	13
12	70	14

Procedure (examples on use of 3 and 5 Standards, single):

1. Calculate the number of wells required for the assay and mark them on the White Microtiter Plate; consider covering wells not to be used to avoid sample contamination. After use, dry the White Plate and return to the ziplock bag for future use.

   Note: *There is a possibility to use 3,4 or all 5 Standards available in the kit in singlets or in dublets(see Quick guide for suPARnostic Standard ELISA Kit), depending on the users needs.*

2. Calculate the number of wells required for the assay. Carefully cut open the foil pouch and remove the Clear Microtiter strip Plate. Remove extra wells from the Clear coated plate and return the unused wells to the foil pouch with desiccant, seal and return to 4˚C storage for future use.



Note: *On completion of the assay retain both remaining wells and the plate frames for future use, if less than the full plate has been used.*

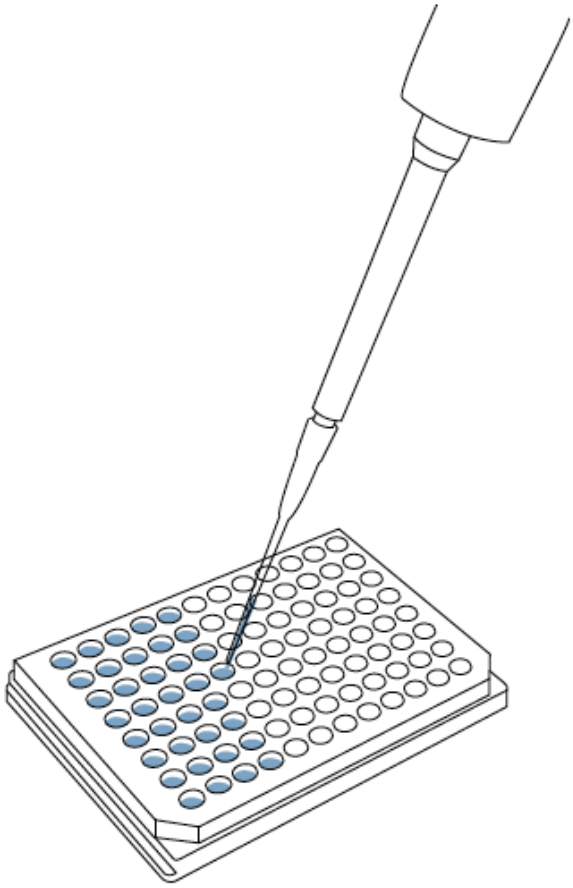
3 a. <u>Three Standards in use:</u>

Transfer 15 µL of the Standard a, b, d (clear cap) to wells A1- C1 in the White mixing plate.

Transfer 15 µL of Dilution Buffer (Component 6) to well D1 in the White mixing plate (Blank).

Transfer 15µL of the Curve Control (blue cap) to well E1 in the White mixing plate.

Transfer 15 µL of each sample to subsequent wells in the White mixing plate as required.



3 b. <u>All Standards in use:</u>

Transfer 15 µL of the Standard a-e (clear cap) to well A1-E1 in the White mixing plate.

Transfer 15 µL of Dilution Buffer (Component 6) to well F1 in the White mixing plate (Blank).

Transfer 15 µL of the Curve Control (blue cap) to well G1 in the White mixing plate.

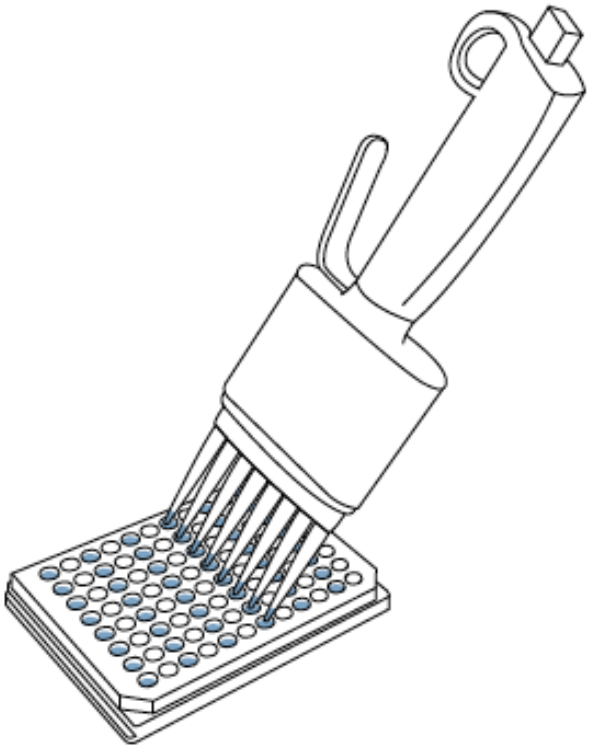
Transfer 15 µL of each sample to subsequent wells in the White mixing plate as required.

Note: *A Standard, Blank and Control must be included with each assay.*
In the case of using 4 Standards, decision about which of Std will be applied in an assay depends on the user; follow the procedure according to the examples above.
For preparing doublets assay follow the 'Standard ELISA Kit Quick guide'.

4. Pipette 135 µL of the Peroxidase Conjugate solution (prepared above) to each used well with a content.

5. Mix gently by slowly pipetting the contents of each well a few times up and down in the pipette tip, then transfer 100 µL to the corresponding well in the Clear coated plate. This can be done using a multichannel pipette. Ensure the pipette tips are changed between each addition.

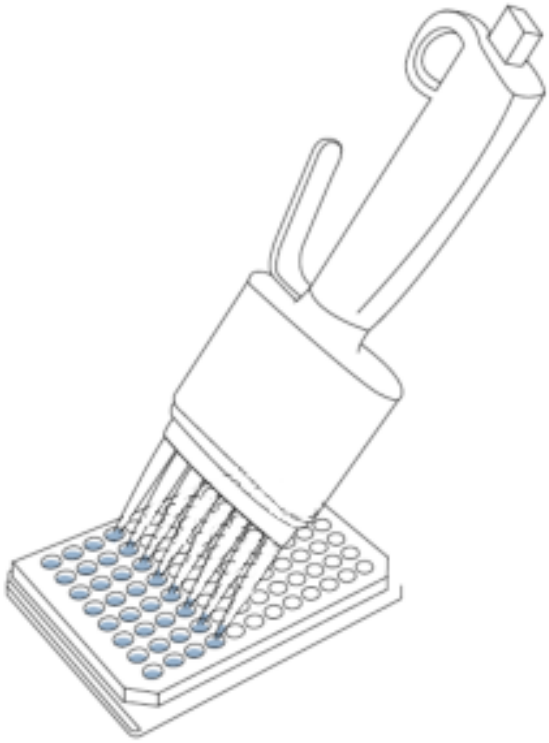
6. Cover the Clear coated plate with Sealing Tape (this may be cut to the correct size prior to use) to prevent evaporation, and incubate for 1 hour at room temperature (18 - 26˚C) in the dark.



10. Remove the Sealing Tape and decant the contents from the wells.

11. Wash wells five times with 250 µL per well of diluted Wash Buffer. This may be done using a multi- or single channel pipette. After emptying the contents of the wells, pipette 250 µL 1x Wash Buffer into the wells and repeat the process a further four times. Alternatively, the Wash Buffer may be gently poured into the wells or added using a squeeze bottle. Tap plate gently onto absorbent paper between each wash step. Carefully blot plate after the final wash and ensure no bubbles remain in the wells.

    Note: *Improper washing will give erroneous results. This step must be carried out carefully. Do not allow the wells to dry out between incubations.*



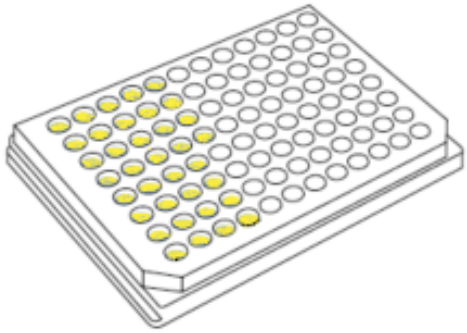
12. Add 100 µL TMB Substrate to each well, cover the Clear coated plate with Sealing Tape and incubate for 20 minutes at room temperature (18 - 26˚C) in the dark.

    Note: *TMB Substrate is easily contaminated. Only remove the required amount for the assay from the bottle (including 10% extra for pipetting margin). Discard unused TMB Substrate. Do not return to bottle.*

13. Remove the Sealing Tape and stop the reaction by adding 100 µL Stop Solution to each well. The colour should change from blue to yellow due to the pH change.

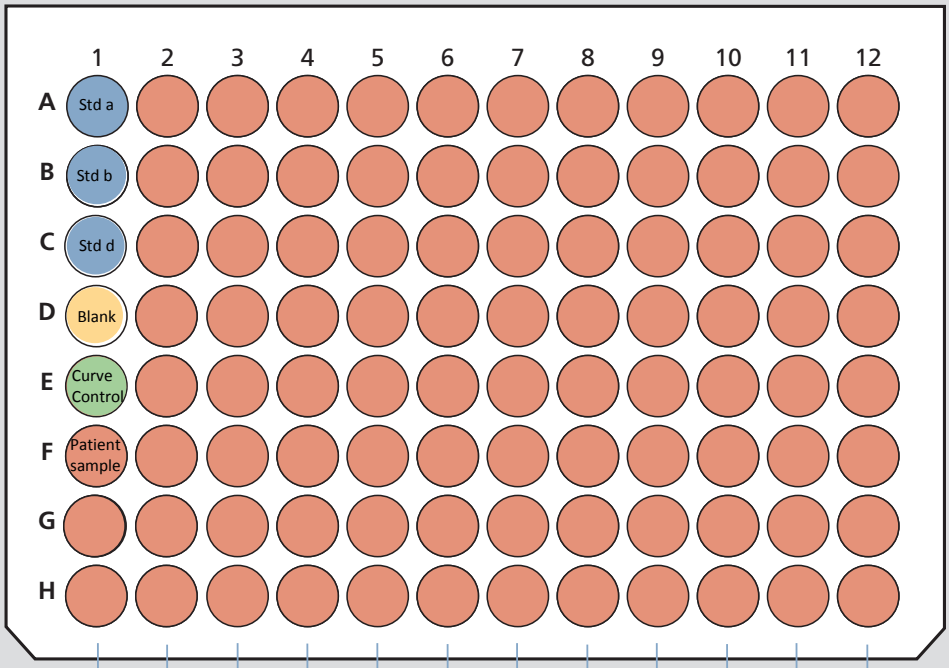
14. Read the absorbance at 450 nm within 30 minutes of stopping the reaction.

    Note: *For dual wavelength readers use a reference filter at approximately 650 nm. Ensure there are no air bubbles in any of the wells. The absorbance of the suPAR standard should be ≥ 1.0 absorbance units.*

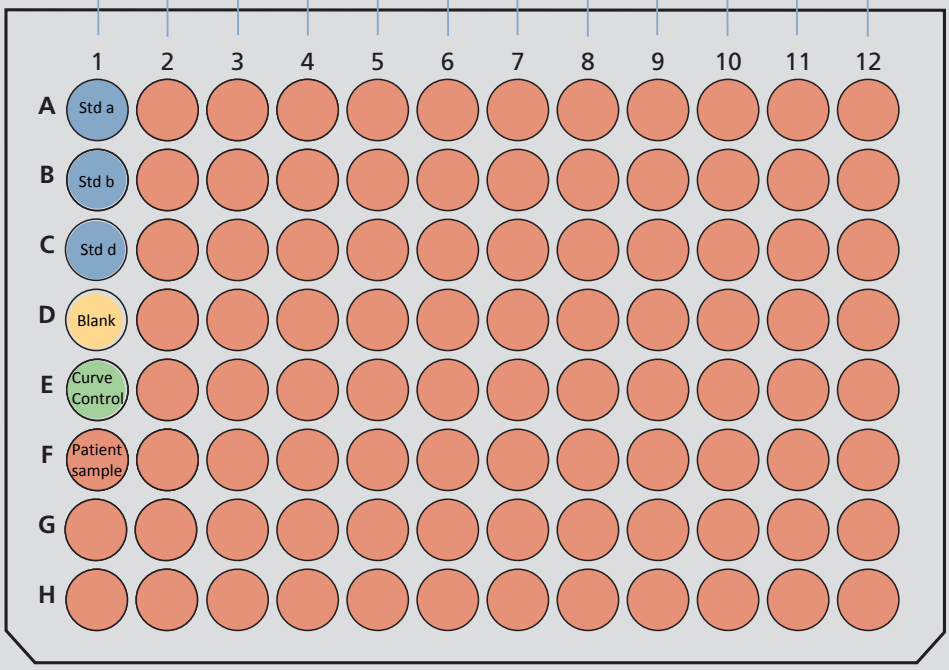


Assay with use of 3 Standards

White Microtiter Plate

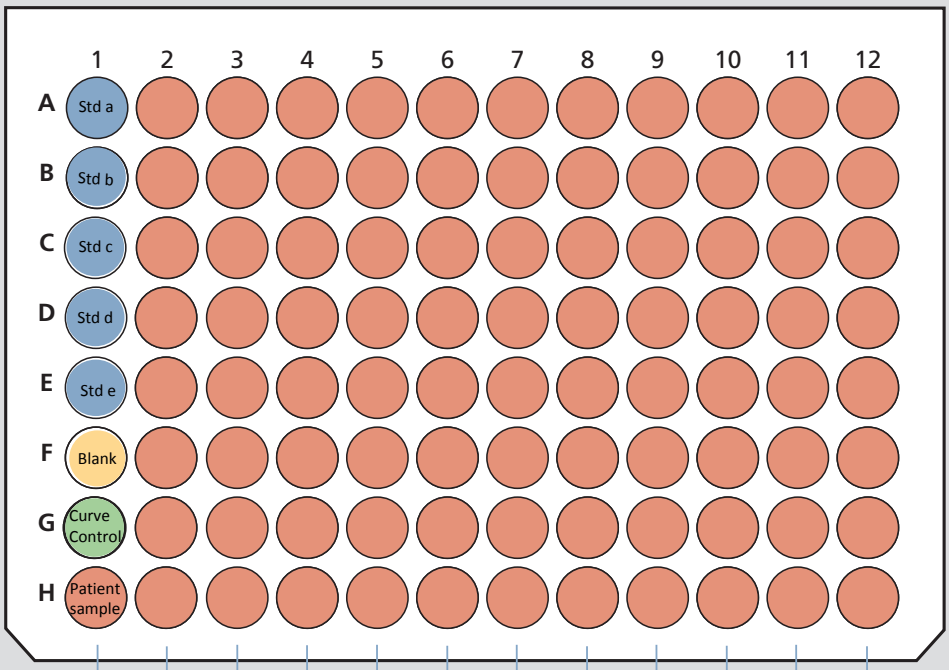


Clear Microtiter Plate



Assay with use of All Standards

White Microtiter Plate



Clear Microtiter Plate