Quick guide for suPARnostic® AUTO Flex ELISA

Preparation of buffers prior to use:

1. Wash buffer working solution:
   Dilute the stock solution by adding 900 mL distilled or deionized water (1:10). The working solution can 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 bottle (empty 15mL bottle in the kit), as described in the table below.

Number of strips	Volume of Conjugate stock (µL)	Volume of Dilution Buffer (mL)
1	5	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 (example on use of 5 Standards in double determination):

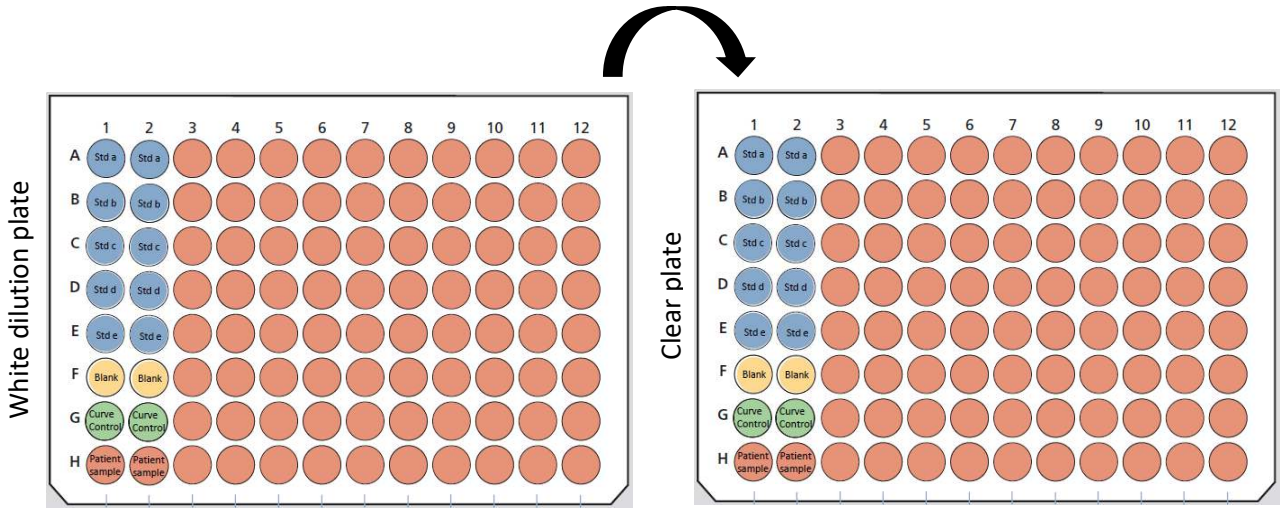
1. Calculate the number of wells required for the assay.
   Mark the wells on the white microtiter plate needed for the experiment and cover the wells not used to avoid sample contamination. After use, dry the white plate and return to the ziplock bag for future use.

2. Carefully 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.

3. Transfer **15 µL of each standard, Blank (buffer), and control,** as shown below, to the white mixing plate.

4. Transfer **15 µL of each sample** to subsequent wells in the White mixing plate as required.
   Note: Standards, Blank, and Curve Control must be included with each run.

5. Pipette **135 µL** of the peroxidase conjugate solution (prepared above) to each used well with a content.



6. Mix the content gently by slowly pipetting up and down a few times in the pipette tip. This should be done using a multichannel pipette.

7. Transfer **100 µL of the mix** to the corresponding well in the clear coated plate. This can be done using a multichannel pipette. Ensure the pipette tips are changed every time.

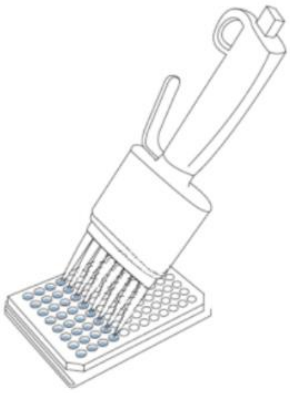
8. Cover the clear coated plate with sealing tape to prevent evaporation, and incubate for **1 hour** at room temperature (18 - 26˚C) in the dark.

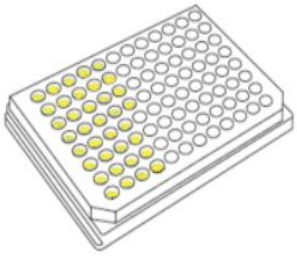
9. Remove the sealing tape and discard the contents from the wells.

10. Wash the wells with 250 µL wash buffer working solution by pipetting up and down a few times. Discard the wash buffer and repeat this process 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.

11. Add **100 µL** TMB substrate to each well and 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 it to bottle.





12. 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.

13. 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 Standard A should be ≥ 1.0 absorbance units.