- Row No. 1 (A-H) is always used for background control and only Washing Buffer is added to each of the wells in this row (3 drops per well).
- Rows No. 2 (A-H) and No. 7 (A-H) are kept empty.
- Incubate the microtiter plate at room temperature (20°C to 30°C) for 2 hrs.
- Wash each microtiter plate well (3x) with 6 drops per well of Washing Buffer.

Step No. 5: Addition of enzyme and its substrate:

- Add 3 drops of reconstituted peroxidase-labelled goat anti-rabbit IgG antibody to each of the microtiter plate wells (vial D).
- Incubate at room temperature (20°C to 30°C) for 2 hrs.
- Wash each microtiter plate well (3x) with 6 drops per well of Washing Buffer.
- Add 3 drops of freshly prepared ABTS-H_2O_2 substrate (vial F) to each well and incubate at room temperature (20°C to 30°C) for 10 to 20 minutes or until standards (Rows 3-6 (A-H)approximate the colours appearing in the reference colour photograph provided.

Step No. 6: Stopping the reaction:

Add 1 drop of stopping reagent (vial P) to each microtiter plate well to stabilize the peroxidase reaction and to stop further darkening of the color.