

Version 1 Last updated 6 May 2020

# ab203359 384-Well SimpleStep ELISA<sup>®</sup> Microplate

384-well microplate can be used as an alternative to the 96-well microplate included with all SimpleStep ELISA kits.

To fully utilize all 384-wells, users need to combine reagents from two 1 x 96 well SimpleStep ELISA kits.

This product is for research use only and is not intended for diagnostic use.

## 1. Technical Hints

- It is recommended that an automated liquid handling system is used with the 384-well microplate. Significant well to well variations can occur if an automated system is not used.
- The 384 well plate is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- For each assay performed, it is recommended that a minimum of two wells be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Please refer to the booklet that accompanies the 1 x 96 well SimpleStep ELISA kit for any target specific information regarding recommended dilution ranges, typical data and troubleshooting.

## 2. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
  - We recommend that you assay all standards, controls and samples in duplicate.
- 2.1 Prepare all reagents, working standards, and samples as directed in the protocol booklet included with the 1 x 96 well SimpleStep ELISA kit.
  - 2.2 Add 25  $\mu\text{L}$  of all sample or standard to appropriate wells.
  - 2.3 Add 25  $\mu\text{L}$  of the Antibody Cocktail to each well.
  - 2.4 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
  - 2.5 Wash each well with 3 x 100  $\mu\text{L}$  1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100  $\mu\text{L}$  1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
  - 2.6 Add 50  $\mu\text{L}$  of TMB Substrate to each well and incubate for time specified in the Assay Procedure section of the of the 1 x 96 well SimpleStep ELISA kit booklet, in the dark on a plate shaker set to 400 rpm.
  - 2.7 Add 50  $\mu\text{L}$  of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm.
  - 2.8 Analyze the data as described in the protocol booklet included with the 1 x 96 well SimpleStep ELISA kit.

## Technical Support

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