ab320051 – HIV1 p24 SimpleStep ELISA® Kit

For the quantitative measurement of HIV1 p24 in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, and monkey serum.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab320051

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pg 10.

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
HIV1 p24 Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
HIV1 p24 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
HIV1 p24 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 5BI	6 mL	10 x 6 mL	+4°C
Cell Extraction Buffer SSW	10 mL	2 x 50 mL	+4°C
Sample Diluent NS	12 mL	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	10 x 96 wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent NSW: Prepare Sample Diluent NSW by diluting Cell Extraction Buffer SSW with Sample Diluent NS. To make 10 mL Sample Diluent NSW combine 8 mL Sample Diluent NS and 2 mL Cell Extraction Buffer SSW. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the p24 standard sample by adding the volume of Sample Diluent NSW indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 19.2 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 364 µL of Sample Diluent NSW into tube number 1 and 150 µL of Sample Diluent NSW into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	20	364	19,200	1,000
2	Standard#1	150	150	1,000	500
3	Standard#2	150	150	500	250
4	Standard#3	150	150	250	125
5	Standard#4	150	150	125	62.5
6	Standard#5	150	150	62.5	31.25
7	Standard#6	150	150	31.25	15.63
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Human Serum	≤ 100%
Human Plasma – Citrate	≤ 100%
Human Plasma – EDTA	≤ 100%
Human Plasma – Heparin	≤ 100%
HEK-293 Cell Culture Supernatant	≤ 100%
Monkey Serum	≤ 100%

Note: The treatment of biological samples with Cell Extraction Buffer SSW, described below, is required to dissociate the viral capsid.

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Consider optional acid-heat treatment of serum described below. Finally, dilute 4 volume parts of samples with one part of Cell Extraction Buffer SSW, incubate for 5 minutes at room temperature. Assay or dilute samples with Sample Diluent NSW and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Consider optional acid-heat treatment of serum described below. Finally, dilute 4 volume parts of samples with one part of Cell Extraction Buffer SSW, incubate for 5 minutes at room temperature. Assay or dilute samples with Sample Diluent NSW and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Consider optional acid-heat treatment of supernatants described below. Finally, dilute 4 volume parts of samples with one part of Cell Extraction Buffer SSW, incubate for 5 minutes at room temperature. Assay or dilute samples with Sample Diluent NSW and assay. Store un-diluted supernatant samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Optional Acid-Heat Treatment Protocol for serum, plasma and cell culture supernatants For the dissociation of immune complex in serum, plasma or cell culture supernatant samples, dilute 90 µL of samples with 60 µL of Sample Diluent NS. Then add 75 µL of 1 N HCL and mix. Incubate 10 minutes at 60°C. Add 75 µL of 1.2 N NaOH + 0.5 M HEPES and mix.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

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.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2024-08-16

ab320051 – HIV1 p24 SimpleStep ELISA® Kit

Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of HIV1 p24.

The standard protein in this kit is full length, recombinant HIV1 p24 of group M, subtype B, strain 92418.

This kit also reacts with recombinant HIV1 p24 from the following subtypes, strains, and isolates:
group M subtype B, strain HXB2
group M subtype B, isolate NY5
group O, strain BCF06

This kit does not react with recombinant HIV1 p24 from the following subtypes and strain:
group M, subtype C, strain 92BR025.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant sample types.

100% pooled human serum, human plasma (citrate, EDTA, Heparin), and monkey serum samples from healthy donors were measured in duplicate. All values were below the detectable range of the assay.

Urine, saliva, milk, CSF, cell extract and tissue extract samples have not been tested with this kit.

For the measurement of p24 in cell extract and tissue extract sample types, use HIV1 p24 ELISA kit ab320051.

CALIBRATION

The NIBSC/WHO unclassified HIV1 p24 international reference reagent 90/636, native p24 protein isolated from detergent treated HIV1, was evaluated in this kit.

The dose response curve of the unclassified HIV1 p24 standard parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep HIV1 p24 kit to approximate NIBSC 90/636 units, use the equation below.

NIBSC (90/636) approximate value (IU/mL) = 0.37 x SimpleStep Species Target value (pg/mL).

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g., linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.046	0.045	0.045
15.63	0.100	0.099	0.100
31.25	0.154	0.153	0.153
62.5	0.259	0.257	0.258
125	0.441	0.439	0.440
250	0.790	0.828	0.809
500	1.497	1.510	1.504
1,000	2.931	2.837	2.884

Table 1. Example of HIV1 p24 standard curve in Sample Diluent NSW. The p24 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 4.3 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Note: Sample concentrations provided in the following sections are prior to treatment with Cell Extraction Buffer SSW or optional Acid-Heat Treatment Protocol.

Recovery

Three concentrations of p24 were spiked into the indicated biological matrix and treated with Cell Extraction Buffer SSW to evaluate signal recovery in the working range of the assay. The interpolated values of the same concentrations of p24 spiked into Sample Diluent NSW were taken as 100%. Samples are assayed without further dilution and measurements are performed in duplicate.

Sample Type	Average % Recovery	Range (%)
100% Human Serum	90	86 - 97
100% Human Plasma – Citrate	90	84 - 98
100% Human Plasma – EDTA	89	82 - 96
100% Human Plasma – Heparin	96	91 - 102
100% HEK-293 Cell Culture Supernatant	95	91 - 103
100% Monkey Serum	83	79 - 89

Acid-Heat Treatment Recovery

Three concentrations of p24 were spiked into the indicated biological matrix and treated with the Acid-Heat Treatment Protocol to evaluate signal recovery in the working range of the assay. The interpolated values of the same concentrations of p24 spiked into Sample Diluent NSW and Acid-Heat treated were taken as 100%. Samples are assayed without further dilution and measurements are performed in duplicate.

Sample Type	Average % Recovery	Range (%)
100% Human Serum	40	38 - 42
100% Human Plasma – Heparin	38	36 - 40
100% HEK-293 Cell Culture Supernatant	108	106 - 110
100% Monkey Serum	37	37 - 38

Acid-Heat Treatment Effect

A single concentration of p24 was spiked into the indicated biological matrix and treated with the Acid-Heat Treatment Protocol or mock-treated to evaluate the effect of the Acid Treatment Protocol. 2-fold serial dilutions of the samples were measured in duplicate. The average, interpolated value of an equivalent serial dilutions of p24 spiked into the indicated biological matrix and mock Acid-Heat treated were taken as 100%.

Sample Type	Average % Effect	Range (%)
100% Human Serum	23	19 - 28
100% Human Plasma – Heparin	21	17 - 26
100% HEK-293 Cell Culture Supernatant	66	56 - 71
100% Monkey Serum	25	25 - 29

Acid-Heat Treatment Recovery of Synthetic Immune Complexes

Three concentrations of p24 were spiked into the indicated biological matrix, pre-incubated with unlabelled capture and detector antibodies, and treated with the Acid-Heat Treatment Protocol to evaluate the signal recovery of p24 complexed with interfering antibodies by the Acid-Heat Treatment Protocol. The signal of three concentrations p24 spiked to the indicated biological matrix, pre-incubated without the addition of the unlabelled antibodies and treated with the Acid-Heat Treatment Protocol were taken as 100%. Samples are assayed without further dilution and measurements are performed in duplicate. There were no detectable signals if the three concentrations of p24 were spiked to the indicated biological matrix, pre-incubated with the unlabelled capture and detector antibodies and mock acid-heat treated, indicating that the unlabelled capture and detector antibodies efficiently formed synthetic immune complexes with p24 and completely blocked the signals.

Sample Type	Average % Effect	Range (%)
100% Human Serum	100	94 - 105
100% Human Plasma – Heparin	98	96 - 101
100% HEK-293 Cell Culture Supernatant	99	93 - 109
100% Monkey Serum	99	91 - 105

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant p24 was spiked into the following biological samples and treated with Cell Extraction Buffer SSW. 2-fold dilution series are made in Sample Diluent NSW.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (EDTA)	100% Human Plasma (Heparin)	100% HEK-293 Super-natant	100% Monkey Serum
Undiluted	pg/mL	863.31	930.20	869.48	1,003.56	972.56	840.24
	% Expected value	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
2	pg/mL	447.31	470.35	457.44	469.38	478.91	415.49
	% Expected value	**104%**	**101%**	**105%**	**94%**	**98%**	**99%**
4	pg/mL	226.12	240.38	251.30	250.96	238.51	228.68
	% Expected value	**105%**	**103%**	**116%**	**100%**	**98%**	**109%**
8	pg/mL	109.93	119.15	116.65	121.88	119.32	114.94
	% Expected value	**102%**	**102%**	**107%**	**97%**	**98%**	**109%**
16	pg/mL	54.64	56.89	55.07	57.13	57.28	61.97
	% Expected value	**101%**	**98%**	**101%**	**91%**	**94%**	**118%**

WHO/NIBSC international reference reagent 90/636 (native p24 protein isolated from detergent treated HIV1) was spiked into the following biological samples and treated with Cell Extraction Buffer SSW. 2-fold dilution series are made in Sample Diluent NSW.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (Heparin)	100% HEK-293 Super-natant	100% Monkey Serum
Undiluted	pg/mL	501.96	570.06	558.77	476.14
	% Expected value	**100%**	**100%**	**100%**	**100%**
2	pg/mL	276.11	272.84	271.68	255.22
	% Expected value	**110%**	**96%**	**97%**	**107%**
4	pg/mL	150.40	140.77	151.13	139.33
	% Expected value	**120%**	**99%**	**108%**	**117%**
8	pg/mL	70.97	65.31	67.72	63.38
	% Expected value	**113%**	**92%**	**97%**	**106%**
16	pg/mL	35.00	35.42	34.34	30.96
	% Expected value	**112%**	**99%**	**98%**	**104%**

Precision

Mean coefficient of variations of interpolated values of p24 from two concentrations of recombinant p24 spiked into HEK-293 cell culture supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.1	7.8

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2024-08-16

DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
HIV1 p24 Capture Antibody 10X	600 µL	+4°C
HIV1 p24 Detector Antibody 10X	600 µL	+4°C
HIV1 p24 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BI	6 mL	+4°C
Cell Extraction Buffer SSW	50 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent NSW: Prepare Sample Diluent NSW by diluting Cell Extraction Buffer SSW with Sample Diluent NS. To make 50 mL Sample Diluent NSW combine 40 mL Sample Diluent NS and 10 mL Cell Extraction Buffer SSW. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent 5BI. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the p24 standard sample by adding the volume of Sample Diluent NSW indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 19.2 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 364 µL of Sample Diluent NSW into tube number 1 and 75 µL of Sample Diluent NSW into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	20	364	19,200	1,000
2	Standard#1	75	75	1,000	500
3	Standard#2	75	75	500	250
4	Standard#3	75	75	250	125
5	Standard#4	75	75	125	62.5
6	Standard#5	75	75	62.5	31.25
7	Standard#6	75	75	31.25	15.63
8	Blank Control	0	75	0	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure for 384-well Plate Format

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 12.5 µL of all sample or standard to appropriate wells.
3. Add 12.5 µL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
6. Add 25 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
7. Add 25 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec – 1 min
Shaking:	Shake between readings

   Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 25 µL Stop Solution to each well and recording the OD at 450 nm.

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Version 1 | 2024-08-16