ab217772 – Human EGF SimpleStep ELISA® Kit

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

For overview, typical data and additional information please visit: www.abcam.com/ab217772

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
Human EGF Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human EGF Detector Antibody (Lyophilized)	1 Vial	10 Vials	+4°C
Human EGF Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Sample Diluent 50BP	20 mL	10 x 20 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 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 50BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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.

20X Detector Antibody: To reconstitute the lyophilized detector antibody, centrifuge the EGF Detector Antibody (Lyophilized) at 10,000 g for 2 minutes. Add 165 µL water and 165 µL Sample Diluent NS, incubate at room temperature for 10 minutes and resuspend well by pipetting.
If the reconstituted antibody will be used within a month, store the unused 20X Reconstituted Detector Antibody at 4°C.
For extended storage, freeze the unused 20X Reconstituted Detector Antibody at -20°C.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 150 µL 20X Detector Antibody and 150 µL Sample Diluent NS with 2.4 mL Antibody Diluent 4BI. 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).

For urine, saliva, milk, and cell culture supernatant samples:

1. Reconstitute the EGF standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 285.7 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 195 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS 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**	105	195	285.7	100
2	Standard#1	150	150	100	50
3	Standard#2	150	150	50	25
4	Standard#3	150	150	25	12.5
5	Standard#4	150	150	12.5	6.25
6	Standard#5	150	150	6.25	3.13
7	Standard#6	150	150	3.13	1.56
8	Blank Control	0	150	0	0

For serum and plasma samples:

1. Reconstitute the EGF standard sample by adding the volume of Sample Diluent 50BP indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 285.7 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 90 µL of Sample Diluent 50BP into tube number 1 and 150 µL of Sample Diluent 50BP 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**	210	90	285.7	200
2	Standard#1	150	150	200	100
3	Standard#2	150	150	100	50
4	Standard#3	150	150	50	25
5	Standard#4	150	150	25	12.5
6	Standard#5	150	150	12.5	6.25
7	Standard#6	150	150	6.25	3.13
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Human Serum	6.25 - 50%
Human Platelet Poor Plasma – Citrate	≤50%
Human Platelet Poor Plasma – EDTA	≤50%
Human Platelet Poor Plasma – Heparin	≤50%
Human Urine	1:4,000 - 1:250
Human Saliva	1:640 – 1:40
Human Milk	1:3,200 – 1:200
Cell Culture Supernatant	≤100%

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. Dilute samples 2-fold into Sample Diluent NS and assay. Any further dilutions should be made in Sample Diluent 50BP. 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 and collect supernatant samples. Since the remaining platelets debris in the supernatant samples can affect the levels of circulating EGF, we strongly recommend preparing platelet-poor plasma. To prepare platelet-poor plasma, centrifuge the supernatants at 10,000 g for 10 min at 4 C and collect the supernatant samples. Dilute samples 2-fold into Sample Diluent NS and assay. Any further dilutions should be made in Sample Diluent 50BP. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute samples at least 1:250 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:40 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Milk Whole or de-fatted milk is compatible with the assay. Milk samples can be de-fatted as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. 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 Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 4a | 21 March 2024

ab217772 – Human EGF SimpleStep ELISA® Kit

Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human EGF.

The standard protein in this kit is the mature full length human EGF.

Native signal was detected in serum, saliva, milk, and urine sample types.

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

Cell extract, and tissue extract samples have not been tested with this kit.

INTERFERENCE
Recombinant human EGFR was prepared at 50 ng/mL and 1 ng/mL and tested for interference. No interference was observed.

SPECIES REACTIVITY
Recombinant mouse EGF was prepared as a serial dilution within the working range of the assay and assayed for cross reactivity. No cross reactivity was observed.

Other species reactivity was determined by measuring 100% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

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	0.056	0.055	0.055
1.56	0.125	0.117	0.121
3.13	0.202	0.181	0.192
6.25	0.371	0.331	0.351
12.5	0.627	0.605	0.616
25	1.166	1.131	1.148
50	2.097	2.264	2.180
100	3.469	3.596	3.533

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

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.066	0.074	0.070
3.13	0.148	0.141	0.144
6.25	0.215	0.204	0.209
12.5	0.314	0.310	0.312
25	0.555	0.501	0.528
50	0.949	0.879	0.914
100	1.759	1.675	1.717
200	3.171	3.286	3.228

Table 2. Example of human EGF standard curve in Sample Diluent 50BP. The EGF standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	12	1.04 pg/mL
Sample Diluent 50BP	17	1.14 pg/mL

Recovery

Three concentrations of EGF were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Human Serum	108	93 - 122
50% Human Platelet Poor Plasma – Citrate	112	100 - 126
50% Human Platelet Poor Plasma – Heparin	109	104 - 114
50% Human Platelet Poor Plasma – EDTA	116	104 - 127
0.2% Human Urine	103	101 - 105
0.25% Human Saliva	92	90 - 96
0.1% Human De-fatted Milk	96	81 - 121
100% Cell Culture Media*	91	88 - 95

*Media is RPMI containing 10% fetal calf serum

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.

Native EGF was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS for urine, saliva, and milk. Sample dilutions are made in Sample Diluent 50BP for serum.

Dilution Factor	Interpolated value	0.4% Human Urine	2.5% Human Saliva	0.5% Human De-fatted Milk	0.5% Human Whole Milk	50% Human Serum
Undiluted	pg/mL	99.8	75.3	101	99.4	57.3
	% Expected value	100	100	100	100	100
2	pg/mL	50.4	33.2	49.4	49.4	26.4
	% Expected value	101	88	98	99	92
4	pg/mL	25.8	15.9	24.5	22.9	11.4
	% Expected value	104	85	97	92	80
8	pg/mL	12.4	8.09	12.1	10.8	5.83
	% Expected value	99	86	96	87	82
16	pg/mL	6.25	4.12	6.19	5.58	NL
	% Expected value	100	88	98	90	NL

NL – Non-Linear

Recombinant human EGF was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent 50BP for plasma and serum samples and Sample Diluent NS for cell culture media.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Platelet Poor Plasma (Citrate)	50% Human Platelet Poor Plasma (EDTA)	50% Human Platelet Poor Plasma (Heparin)	100% Cell Culture Media
Undiluted	pg/mL	240	203	208	207	121
	% Expected value	100	100	100	100	100
2	pg/mL	122	92.9	96.2	96.1	65.7
	% Expected value	102	92	92	93	108
4	pg/mL	61.7	43.1	48.4	44.0	31.0
	% Expected value	103	85	93	85	102
8	pg/mL	31.2	23.9	24.7	23.2	15.7
	% Expected value	104	95	95	90	104
16	pg/mL	15.9	11.4	12.0	10.8	8.02
	% Expected value	106	90	92	84	106

Precision

Mean coefficient of variations of interpolated values of EGF from three concentrations of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	8.0	7.0

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

Version 4a | 21 March 2024

DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human EGF Capture Antibody 10X	600 µL	+4°C
Human EGF Detector Antibody (Lyophilized)	1 Vial	+4°C
Human EGF Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent 50BP	20 mL	+4°C
Sample Diluent NS	250 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 50BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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.

20X Detector Antibody: To reconstitute the lyophilized detector antibody, centrifuge the EGF Detector Antibody (Lyophilized) at 10,000 g for 2 minutes. Add 165 µL water and 165 µL Sample Diluent NS, incubate at room temperature for 10 minutes and resuspend well by pipetting.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 300 µL 20X Detector Antibody and 300 µL Sample Diluent NS with 4.8 mL Antibody Diluent 4BI. 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).

For urine, saliva, milk, and cell culture supernatant samples:

1. Reconstitute the EGF standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 285.7 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 130 µL of Sample Diluent NS into tube number 1 and 75 µL of Sample Diluent NS 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**	70	130	285.7	100
2	Standard#1	75	75	100	50
3	Standard#2	75	75	50	25
4	Standard#3	75	75	25	12.5
5	Standard#4	75	75	12.5	6.25
6	Standard#5	75	75	6.25	3.13
7	Standard#6	75	75	3.13	1.56
8	Blank Control	0	75	0	0

For serum and plasma samples:

1. Reconstitute the EGF standard sample by adding the volume of Sample Diluent 50BP indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 285.7 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 60 µL of Sample Diluent 50BP into tube number 1 and 75 µL of Sample Diluent 50BP 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**	140	60	285.7	200
2	Standard#1	75	75	200	100
3	Standard#2	75	75	100	50
4	Standard#3	75	75	50	25
5	Standard#4	75	75	25	12.5
6	Standard#5	75	75	12.5	6.25
7	Standard#6	75	75	6.25	3.13
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.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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Version 4a | 21 March 2024