Triclosan

• Intended Use

For detection of Triclosan and Triclosan methyl. Please refer to the attached specific procedures for water (groundwater, surface water, well water, effluent), and soil. Application procedures for other sample matrices can be obtained from Abraxis.

• Principle

The Abraxis Triclosan Microtiter Plate Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of Triclosan. In the assay system, standards, controls, or samples are added, along with an antibody specific to Triclosan, to microtiter wells coated with Goat Anti-Rabbit Antibody and incubated for thirty (30) minutes. The Triclosan enzyme conjugate is then added. At this point, a competitive reaction occurs between the Triclosan, which may be in the sample, and the enzyme-labeled Triclosan analog for the antibody binding sites on the microtiter well. The reaction is allowed to continue for thirty (30) minutes. After a washing step, the presence of Triclosan is detected by adding the "Color Solution," which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled Triclosan bound to the Triclosan antibody catalyzes the conversion of the substrate/chromogen mixture to a colored product. The color reaction is stopped and stabilized after a twenty (20) minute incubation period by the addition of diluted acid (stopping solution). The color is then evaluated using an ELISA reader.

A dose response curve of absorbance vs. concentration is generated using results obtained from the standards. The concentration of Triclosan present in the control and samples is determined directly from this curve. Since the labeled Triclosan (conjugate) was in competition with the unlabeled Triclosan (sample) for the antibody sites, t**he intensity of the color developed is inversely proportional to the concentration of Triclosan present in the sample.**

• Reagents

The Abraxis Triclosan Plate Kit contains the following items:

1. Microtiter Plate coated with Goat-Anti Rabbit Antibody

96 test kit: 12 strips of 8 antibody coated wells and strip holder (1).

2. Triclosan Antibody Solution
Triclosan antibody (rabbit anti-Triclosan) solution in a colored (red) buffered saline solution with preservative and stabilizers.

96 test kit: One vial containing 6 mL

3. Triclosan Enzyme Conjugate
Horseradish peroxidase (HRP) labeled Triclosan analog in a colored (green) buffered solution with preservative and stabilizers.

96 test kit: One vial containing 6 mL

4. Triclosan Standards
Seven concentrations (0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 ppb) of Triclosan standards in distilled water with preservative and stabilizers.

96 test kit: Each vial contains 1.0 mL

5. Control
A concentration (approximately 0.75 ppb) of Triclosan in distilled water with preservative and stabilizers.

96 test kit: One vial containing 1.0 mL

6. Diluent/Zero Standard (Sample Diluent)
Distilled water with preservative and stabilizers without any detectable Triclosan.

96 test kit: One bottle containing 30 mL

7. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethyl benzidine in an organic base.

96 test kit: One bottle containing 16 mL

8. Stopping Solution
A solution of diluted acid.

96 test kit: Two bottles containing 6 mL each

9. Washing Buffer (5x) Concentrate
Buffered salts with detergent and preservatives.

96 test kit: One bottle containing 100 mL

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box.

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Precision pipets capable of delivering 50, 100 and 250 uL, and tips*

Tape or Parafilm®*

Timer*

Distilled or deionized water for diluting Wash Buffer

Storage bottle with 1000 mL capacity for storage of 1x Wash Buffer*

Microplate or strip reader capable of reading absorbance at 450 nm*

* Please contact Abraxis for supplier information.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the Triclosan concentration of a sample exceeds 2.5 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 uL of the sample to 900 uL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor, e.g. 10.

The presence of the following substances up to 1,000 ppm were found to have no significant effect on the Triclosan Plate Assay results: phosphate, sodium fluoride, sodium chloride, magnesium and humic acid. Nitrate, manganese, calcium, sodium thiosulfate and sulfate up to 10,000 ppm. Copper, iron and zinc up to 100 ppm.

• Reagent Preparation

All reagents must be allowed to come to room temperature.

Wash Buffer

In a 1000 mL container, dilute the wash buffer concentrate 1:5 by the addition of distilled or deionized water (i.e., 100 mL of wash buffer concentrate plus 400 mL of H_2O). This solution is used to wash the antibody coated wells.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each well in an identical manner.

Add reagents directly to the bottom of the well while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

The microtiter plate consists of 12 strips of 8 wells. If fewer than twelve strips are used, remove the unneeded strips and store refrigerated in the resealable foil bag (with desiccant) provided.

If more than 3 strips are being used per run, the use of a multi-channel pipette is recommended for the addition of conjugate, antibody, color, and stopping solutions.

Do not use any reagents beyond their stated shelf life. Each component used in any one assay should be of the same lot number and stored under identical conditions.

Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis Triclosan Plate Assay will detect Triclosan, Triclosan methyl and related compounds. Refer to the specificity table for data on several related compounds. The Abraxis Triclosan Plate Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some

action should be confirmed by an alternative method.

• Quality Control

A control solution at approximately 0.75 ppb of Triclosan is provided with the Abraxis Triclosan Plate Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

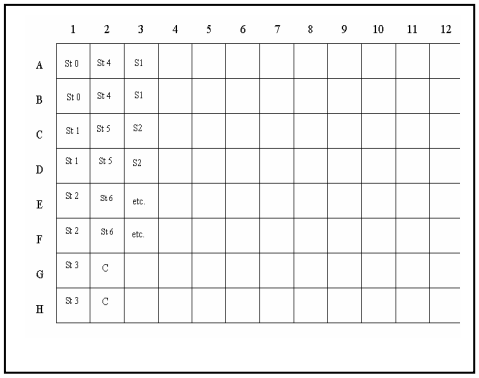
• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

St0-St6: Standards

C: Control

S1-Sx: Samples



1. Add 50 uL of the appropriate standard, control, or sample. Analysis in duplicates or triplicates is recommended.
2. Add 50 uL of Triclosan antibody solution successively to each well. Cover wells with parafilm or tape to prevent contamination and evaporation. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Be careful not to spill the contents. Incubate at ambient temperature for 30 minutes.
3. After the incubation, add 50 uL of Triclosan enzyme conjugate solution successively to each well. Cover wells with parafilm or tape and thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds. Incubate at ambient temperature for 30 minutes.
4. After the incubation, carefully remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips with the diluted Wash Buffer (see Reagent Preparation) by adding a volume of at least 250 uL of Wash Buffer to each well. Vigorously shake the contents of the wells into a waste container. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels. Repeat this wash step two times, for a total of 3 rinses.
5. Add 100 uL of Color Solution successively to each well. Cover wells with parafilm or tape. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for a full 20-30 seconds Incubate at ambient temperature for 20 minutes.
6. Add 50 uL of Stopping Solution successively to each well.
7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameter or alternatively point to point). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the %B/Bo for each standard by dividing the mean absorbance value for each standard by the mean absorbance value for the Diluent/Zero Standard (Standard 0). Construct a standard curve by plotting the %B/Bo for each standard on the vertical linear (Y) axis versus the corresponding Triclosan concentration on the horizontal log (X) axis on the graph paper provided. Calculate the %B/Bo for the control and sample(s) and obtain the concentration of Triclosan (in ppb) by interpolation using the constructed standard curve.

Samples exhibiting a concentration lower than 0.020 ppb should be assumed to be below the detection limit of the assay. Samples exhibiting a concentration higher than 2.5 ppb must be diluted to obtain accurate results.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppb)	0.097	0.236	0.926
% CV (within assay)	9.7	7.3	8.3
% CV (between assay)	12.4	12.4	9.6

Limit of Detection

The Abraxis Triclosan Plate Assay has an estimated minimum detection concentration based on a 90% B/Bo of 0.020 parts per billion (ppb).

Recovery

Four (4) groundwater samples were spiked with various levels of Triclosan and then assayed using the Abraxis Triclosan Plate Assay. The following results were obtained:

Amount of Triclosan Added (ppb)	Recovery Mean (ppb)	Recovery S.D. (ppb)	Recovery %
0.5	0.467	0.029	93
1	1.122	0.079	112
2	2.146	0.082	107
Average			104

Sensitivity

The Abraxis Triclosan Plate Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 20 ppt (0.020 ppb). Refer to appropriate application notes or procedures for sensitivity in specific sample matrices.

Specificity

The cross-reactivity of the Abraxis Triclosan Plate Assay for various related and unrelated compounds can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required to displace 50% (50% B/Bo).

B/Bo Compound	LDD (ppb)	50% (ppb)
Triclosan	0.020	0.250
Triclosan methyl	0.015	0.080
PBDE Congener 28	0.034	0.61
PBDE Congener 47	0.020	0.390
PBDE Congener 49	5.2	17.8
PBDE Congener 99	2.15	15.0
4'-OH-BDE-47	0.13	7.8
5-OH-BDE-47	0.15	5.6
6-OH-BDE-47	0.66	10.2
2,4,5-Tribromobiphenyl	>100	>100
2,4',5-Tribromobiphenyl	54	9,100
2,3,7,8-Tetrachloro-dibenzo-p-dioxin	>100	>100
T3	0.94	40
L-Thyroxine (T4)	340	700

The following compounds demonstrated no reactivity in the Triclosan Plate Assay at concentrations up to 1,000 ppb: Biphenyl, 2,4-D.

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
54 Steamwhistle Drive
Warminster, PA 18974
(215) 357-3911 * Fax (215) 357-5232

• Ordering Information

Abraxis Triclosan Assay Kit, 96T	PN	530114
Triclosan Sample Diluent	PN	530112

• General Limited Warranty

Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

080808

Triclosan in Water

• Intended Use

For detection of Triclosan and Triclosan methyl in water samples: groundwater, surface water, well water, effluent.

• Materials Required but Not Provided

Methanol (HPLC Grade or equivalent).

• Sample Information

Water samples should be collected in glass vessels (teflon in the cap liners). **Immediately** upon collection, methanol (HPLC grade) should be added to the samples (25% v/v final concentration of methanol) to prevent adsorptive losses to the glass containers.

After samples are diluted, those samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

• Procedural Notes and Precautions

Prepare water samples as described above. Follow the assay procedure as described in the Triclosan Abraxis Assay kit package insert.

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical Ln (Y) axis versus the corresponding Triclosan concentration on horizontal Linear (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppt of Triclosan by interpolation using the standard curve.

(Contact Abraxis for detailed application information on specific photometers.)

Photometric Analyzer

Some instrument manufacturers make available photometers allowing for calibration curves to be automatically calculated and stored. Refer to instrument operating manual for detailed instructions. To obtain results for the Abraxis Triclosan Assay on instruments allowing data tranformation the following parameter settings are recommended:

Multiply the sample and control results by a factor of 1.33 to account for the initial dilution of sample with methanol or alternatively program the Photometric Analyzer as listed below to automatically correct for the dilution factor.

Data Reduct	:	Lin. Regression
Xformation	:	Ln / Linear
Read Mode	:	Absorbance
Wavelength	:	450 nm
Units	:	PPT
# Rgt Blk	:	0

Calibrators:

# of Cals	:	4
# of Reps	:	2

Concentrations:

#1:	0.00	PPT
#2:	25	PPT
#3:	100	PPT
#4:	1000	PPT

Range	:	20 - 1000
Correlation	:	0.990
Rep. %CV	:	10%

• Performance Data

Sensitivity

The Triclosan Assay has an estimated minimum detectable concentration in water, based on a 90% B/Bo of 20 ppt (0.020 ppb).

Recovery

Four (4) samples, including a municipal water source, drinking water from a local well, a sample from a local pond and a small creek were spiked with various levels of Triclosan , diluted with methanol, and then assayed using the Triclosan Assay. The following results were obtained:

Amount of Triclosan Added (ppt)	Recovery Mean (ppt)	Recovery S.D. (ppt)	Recovery %
62.5	52.2	4.8	83.4
125	109.1	10.8	87.3
250	252.5	9.1	101.0
500	565.6	28.4	113.1
Average			96.2

Precision

The following results were obtained:

Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppt)	40	124	519
% CV (within assay)	7.5	5.9	4.7
% CV (between assay)	11.4	10.4	6.2

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
54 Steamwhistle Drive
Warminster, PA 18974
(215) 357-3911 * Fax (215) 357-5232

• Availability

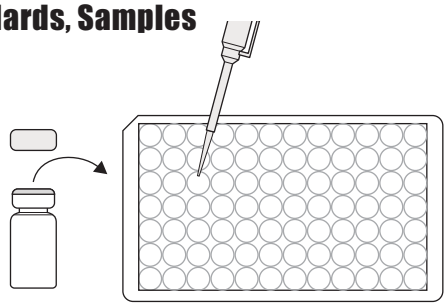
Abraxis Triclosan Assay Kit, 100T	PN	530111
Triclosan Sample Diluent	PN	530112

022306

Triclosan Plate, Detailed ELISA Procedure

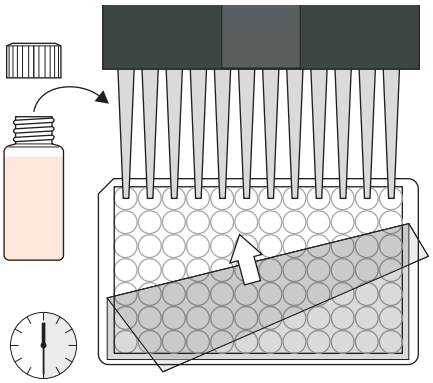
1. Addition of Standards, Samples

Add 50 uL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



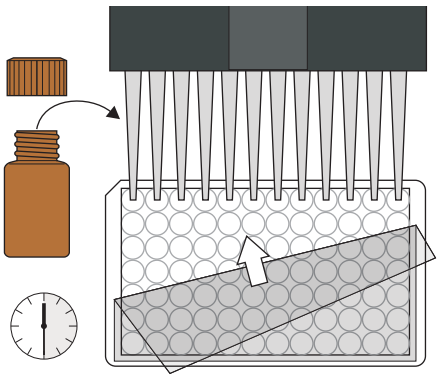
2. Addition of Antibody Solution

Add 50 uL of the Triclosan anitbody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



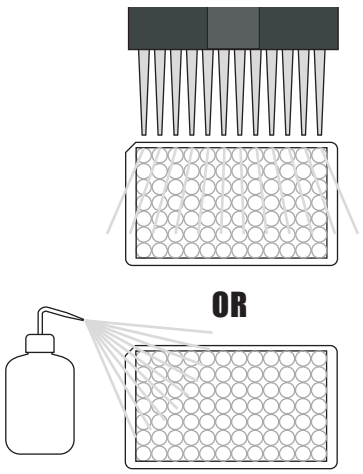
3. Addition of Enyzme Conjugate

Add 50 uL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



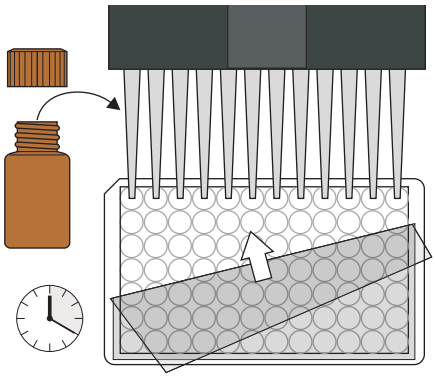
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or wash bottle using the diluted 1X washing buffer solution. Please use at least a volume of 250 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



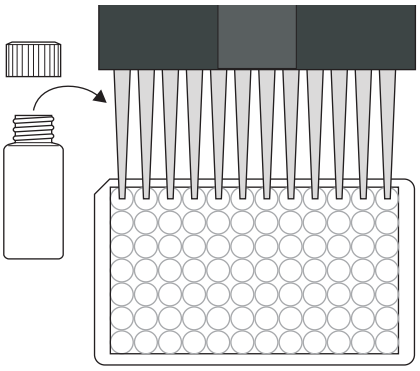
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 20 min at room temperature.



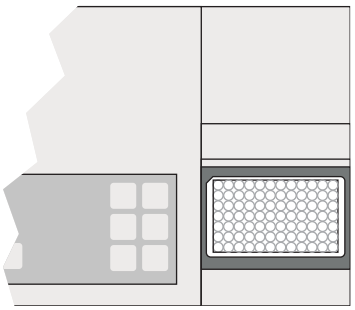
6. Addition of Stopping Solution

Add 50 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.

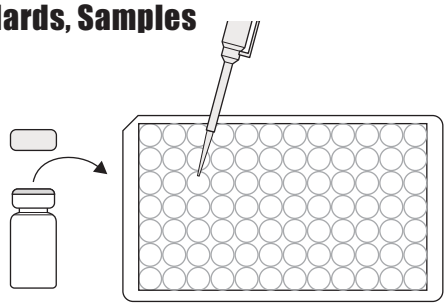


For Ordering or Technical Assistance Contact:
ABRAXIS, LLC
54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

Triclosan Plate, Concise ELISA Procedure

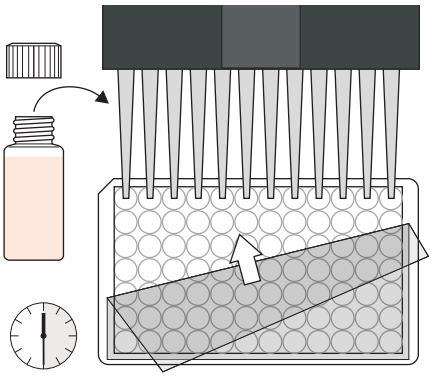
1. Addition of Standards, Samples

Add 50 uL of standard solutions, control or samples.



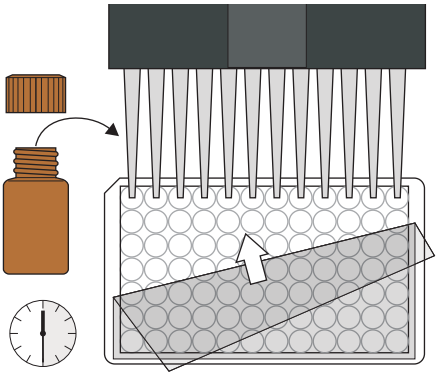
2. Addition of Antibody Solution

Add 50 uL of antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



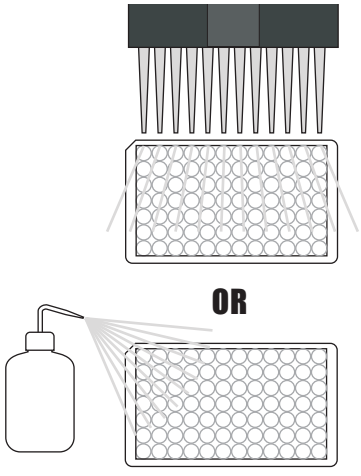
3. Addition of Enyzme Conjugate

Add 50 uL of the enyzme conjugate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



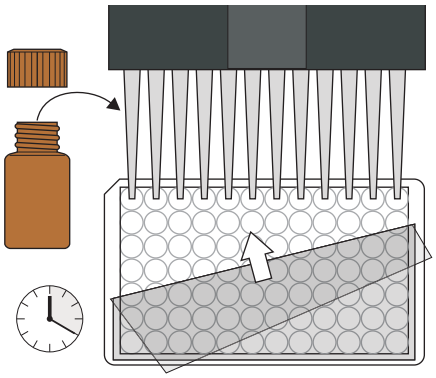
4. Washing of Plates

Wash the plates three times with 250 uL of diluted 1X washing buffer.



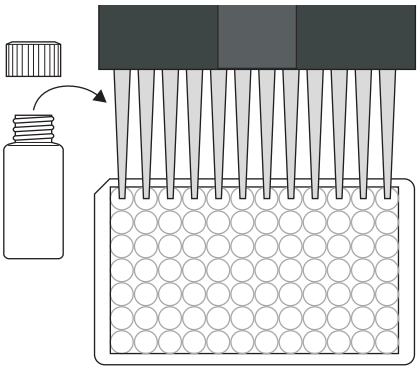
5. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 20 minutes at room temperature and away from direct sunlight.



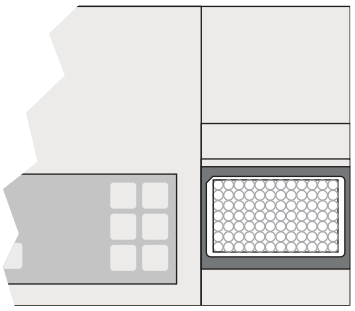
6. Addition of Stopping Solution

Add 50 uL of stop solution.



7. Measurement of Color

Measure color at 450 nm. Calculate results.



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