

**FOR INFORMATION ONLY.  
WHEN PERFORMING  
THE ASSAY ALWAYS REFER  
TO PACKAGE INSERT  
SUPPLIED WITH THE KIT**



## CYFRA 21-1 EIA

Prod. No. 211-85













Instructions for use

Enzyme immunometric assay kit.

2009-10

For 96 determinations

*For Research Use Only-Not for use in diagnostic procedures*

 Use By	 Temperature limitation
 Batch code	 Consult Instructions for Use
 Date of manufacture	 Biological risks
 Catalogue number	 Contents of kit
 Manufacturer	 From mouse
 Contains sufficient for <96> tests	 Human origin

### INTENDED USE

The CYFRA 21-1 EIA kit is intended for the quantitative determination of soluble cytokeratin 19 fragments in human serum.

### SUMMARY AND EXPLANATION OF THE ASSAY

Cytokeratin 19 is a member of a family of at least twenty different cytokeratin polypeptides. Cytokeratins form the intermediate filament structure of epithelial cells (1, 2). Cytokeratin filaments are poorly soluble but following proteolytic degradation, soluble cytokeratin fragments are formed and released into body fluids.

CYFRA 21-1 is an immunoassay that determines the level of cytokeratin 19 fragments in serum (3-6). The CYFRA 21-1 EIA is based on two monoclonal antibodies (BM 19.21 and KS 19.1) specific for cytokeratin 19 (3, 7-8). Elevated levels of cytokeratin 19 fragments are seen in serum from patients with lung cancer (5, 9-12) and also in other cancers eg. bladder cancer (13).

## **PRINCIPLE OF THE TEST**

The CYFRA 21-1 EIA is a solid phase, non-competitive immunoassay based on two monoclonal antibodies (derived from mice) directed against two separate antigenic determinants of soluble fragments of cytokeratin 19 (7-8). Calibrators, controls and unknown samples are incubated together with biotinylated Anti-CYFRA 21-1 MAb and horseradish peroxidase (HRP) labelled Anti-CYFRA 21-1 MAb in streptavidin coated microtiter strips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour development is proportional to the amount of CYFRA 21-1 present in the samples.

The colour intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution).

Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The CYFRA 21-1 concentrations of unknown samples are then read from the calibration curve.

## **REAGENTS**

- Each CYFRA 21-1 EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8°C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

Component	Quantity	Storage and stability after first use
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**MICROPLA**

<b>Streptavidin Microplate</b>	1 Plate	2-8°C until expiry date stated on the plate
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12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminum pouch, containing desiccant. Reseal carefully to keep dry.

**CAL CYFRA 21-1 A**

<b>CYFRA 21-1 Calibrator A</b>	1 x 8 mL	2-8°C until expiry date stated on the vial
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Phosphate buffered salt solution containing bovine serum albumin, an inert yellow dye, and a non-azide antimicrobial preservative. Ready for use. Should also be used for dilution of samples.

<b>CYFRA 21-1 Calibrators B-F</b>	5 vials, lyophilized	Stability after reconstitution 4 weeks at 2-8°C 4 months at -20°C or below
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**CAL CYFRA 21-1 B**

1 x 1 mL

**CAL CYFRA 21-1 C**

1 x 1 mL

**CAL CYFRA 21-1 D**

1 x 1 mL

**CAL CYFRA 21-1 E**

1 x 1 mL

**CAL CYFRA 21-1 F**

1 x 1 mL

The lyophilized calibrators contain CYFRA 21-1 antigen in a phosphate buffered salt solution containing bovine serum albumin, an inert yellow dye, and a non-azide antimicrobial preservative. To be reconstituted with distilled or deionized water before use.

**NOTE:** The exact CYFRA 21-1 concentration is lot specific and is indicated on the label of each vial.

Component	Quantity	Storage and stability after first use
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<b>CYFRA 21-1 Controls</b>	2 vials, lyophilized	Stability after reconstitution 1 week at 2-8°C 4 months at -20°C or below
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CONTROL	CYFRA 21-1	1	1 x 1 mL
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CONTROL	CYFRA 21-1	2	1 x 1 mL
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The lyophilized controls contain CYFRA 21-1 antigen in a human serum matrix and a non-azide antimicrobial preservative. To be reconstituted with distilled or deionized water before use.

BIOTIN	Anti-CYFRA 21-1
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<b>Biotin Anti-CYFRA 21-1</b>	1 x 15 mL	2-8°C until expiry date stated on the vial
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Biotin Anti-CYFRA 21-1 monoclonal antibody from mouse,, approximately 1.25 µg/mL. Contains Tris-HCl buffered salt solution (pH 7.2), bovine serum albumin, blocking agents, detergent, an inert blue dye, and a non-azide antimicrobial preservative. To be mixed with Tracer before use.

CONJ	Anti-CYFRA 21-1
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<b>Tracer, HRP Anti-CYFRA 21-1</b>	1 x 0.75 mL	2-8°C until expiry date stated on the vial
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Stock Solution of HRP Anti-CYFRA 21-1 monoclonal antibody from mouse, approximately 42 µg/mL. Contains non-azide antimicrobial preservatives. To be mixed with Biotin Anti-CYFRA 21-1 prior to use.

SUBS	TMB
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<b>TMB HRP-Substrate</b>	1 x 12 mL	2-8°C until expiry date stated on the vial
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Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine (TMB). Ready for use.

STOP
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<b>STOP Solution</b>	1 x 15 mL	2-8°C until expiry date stated on the vial
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Contains 0.12 M hydrochloric acid. Ready for use.

Component	Quantity	Storage and stability after first use
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<b>WASHBUF</b>	<b>25X</b>	
<b>Wash Concentrate</b>	1 x 50 mL	2-8°C until expiry date stated on the bottle

A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative.

To be diluted with distilled or deionized water 25 times before use.

#### Indications of instability

The TMB HRP-Substrate should be colorless or slightly bluish. A blue color indicates that the reagent has been contaminated and should be discarded.

## WARNINGS AND PRECAUTIONS

#### For Investigational Use Only:

**The performance characteristics of this product have not been established.**

- Follow the instructions in the Package insert. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Handle all patient specimens as potentially infectious. It is recommended that human source reagent and human specimens be handled in accordance with the OSHA Standard on Bloodborne pathogens (14). Biosafety level 2 (15) or other appropriate biosafety practices should be used for material that contain or are suspected of containing infectious agents.
- Avoid contact with reagents containing hydrogen peroxide or hydrochloric acid. In case of contact with any of these reagents, wash thoroughly with water.
- Follow local guidelines for disposal of all waste material.

#### Caution

Each donor unit used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV-1/2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

## **SPECIMEN COLLECTION AND HANDLING**

The CYFRA 21-1 EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2-8°C for 1 day. For longer periods store samples at -40°C or below. Avoid repeated freezing and thawing of the samples. If aliquoted choose the right sized tube i.e. limit the empty space in the tube. Bring frozen samples to room temperature and mix thoroughly by gentle inversion before analysis. **Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.**

## **PROCEDURE**

### **Materials required but not supplied with the kit**

#### **1. Microplate shaker**

Shaking should be medium to vigorous, approximately 900-1100 oscillations/min.

#### **2. Microplate wash device**

Automatic plate wash capable of performing 1 and 6 washing cycles with a minimal fill volume of 350 µL/well/washcycle.

The Nunc Immuno-8 manual strip washer is recommended if an automatic microplate washer is not used.

#### **3. Microplate spectrophotometer**

With a wavelength of 620 nm and/or 405 nm, and an absorbance range of 0 to 3.0.

#### **4. Precision pipettes**

With disposable plastic tips for dispensing microliter volumes. An 8-channel pipette or dispenser pipette with disposable plastic tips for delivery of 100 µL is recommended but not required. Pipettes for dispensing milliliter volumes.

#### **5. Distilled or deionised water**

For reconstitution of CYFRA 21-1 Calibrators, CYFRA 21-1 Controls and for preparation of diluted wash solution.

### **Procedural notes**

1. A thorough understanding of this package insert is necessary to ensure proper use of the CYFRA 21-1 EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25°C) prior to use. Frozen specimens must be gently but thoroughly mixed by gentle inversion after thawing. **Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.** If aliquoted choose the right sized tube i.e. limit the empty space in the tube.

3. Before starting to pipette calibrators and unknown specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. **In order to ensure efficient washing make sure that all wells are completely filled to the top edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.**
  - Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. **It's highly recommended to use STRIP process mode and OVERFLOW wash mode with a dispensing volume of 800 µL.** The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.
5. The TMB HRP-Substrate is very sensitive to contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial into a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or dispenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper precision pipetting technique when handling samples and reagents. Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over. A diligent pipetting technique is of particular importance when handling the samples and the TMB HRP-Substrate solution.

Preparation of reagents	Stability of prepared reagent
<b>CYFRA 21-1 Calibrators</b>	4 weeks at 2-8°C 4 months at -20°C or below

Add exactly 1.0 mL of distilled water to each vial. Allow standing for at least 15 minutes to reconstitute and mix gently before use. NOTE: The concentration of the calibrators is stated on the labels and should be used for calculation of the results. Mix only by gentle swirling or inversion. **Do Not Vortex.**

<b>Preparation of reagents</b>	<b>Stability of prepared reagent</b>
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<b>CYFRA 21-1 Controls</b>	1 week at 2-8°C 4 months at -20°C or below
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Add exactly 1.0 mL of distilled water to each vial. Allow standing for at least 15 minutes to reconstitute and mix gently before use. NOTE: The range of the controls is stated on the labels. Mix only by gentle swirling or inversion. **Do Not Vortex.**

<b>Wash Solution</b>	2 weeks at 2-25°C in sealed container
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Pour the 50 mL Wash Concentrate into a clean container and dilute 25-fold by adding 1200 mL of distilled or deionised water to give a buffered Wash Solution.

<b>Antibody Solution</b>	1 day at 2-8°C
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Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-CYFRA 21-1 with 1 mL of Biotin Anti-CYFRA 21-1 per strip (see table below):

<b>No. of Strips</b>	<b>Tracer, HRP Anti-CYFRA 21-1 (µL)</b>	<b>Biotin Anti-CYFRA 21-1 (mL)</b>
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass bottle for preparation of Antibody Solution.

## ASSAY PROCEDURE

Perform each determination in duplicate for calibrators, controls and unknown samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20-25 °C) before use.

1. Start to prepare CYFRA 21-1 Calibrators, Controls 1 & 2, Wash Solution and Antibody Solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Mix samples by gentle inversion. **Do not Vortex for more than 1 second.** Pipette 50 µL of the CYFRA 21-1 Calibrators (CAL A, B, C, D, E, and F), Controls 1 & 2 and unknown specimens (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	1 st Unk				
B	Cal A	Cal E	1 st Unk				
C	Cal B	Cal F	2nd Unk				
D	Cal B	Cal F	2nd Unk				
E	Cal C	C1					
F	Cal C	C1					
G	Cal D	C2					
H	Cal D	C2					

4. Add 100 µL of Antibody Solution to each well using a 100 µL 8-channel precision pipette (or a 100 µL precision pipette). Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over.
5. Incubate the plate for 1 hour ( $\pm$  5 min) at room temperature (20-25°C) with constant shaking of the plate using a microplate shaker.
6. After the incubation aspirate and wash each strip 6 times.
7. Add 100 µL of TMB HRP-Substrate to each well using the same procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between addition to the first and last well should not exceed 5 min.
8. Incubate for 30 min ( $\pm$  5 min) at room temperature with constant shaking. Avoid exposure to direct sunlight.
9. Immediately read the absorbance at 620 nm in a microplate spectrophotometer.

### **Option**

If the laboratory does not have access to a microplate spectrophotometer capable of reading at 620 nm the absorbance can be determined as in the alternative item 9 below:

Alt. 9. Add 100  $\mu$ L of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

### **Measurement range**

The CYFRA 21-1 EIA measures concentrations between 0.5 and approximately 50 ng/mL. If CYFRA 21-1 concentrations above the measuring range are expected, it is recommended to dilute samples with CYFRA 21-1 Calibrator A prior to analysis (see "Calculation of results with diluted samples").

### **Quality control**

CYFRA 21-1 Control 1 and 2 should be used for validation of each assay series. Ranges of expected results are indicated on the vial labels.

The CYFRA 21-1 assay results should be considered valid if:

- The mean values of control duplicates are within the specified ranges.
- The duplicate replicates of calibrators B-F and controls do not exceed a CV of 15%.
- The duplicate replicates of calibrator A (zero) are not more than 0.06 OD units different from each other.

If an assay results in invalid calibrator or control results, a complete check of reagents, accuracy of pipettes, plate washer and reader performance should be made and the analysis repeated. Each laboratory may also prepare its own serum pools at different levels, which can be used as internal controls in order to assure the precision of the assay.

### **Reference materials**

Since no common reference material is available for CYFRA 21-1 antigen, CYFRA 21-1 EIA Calibrator values are assigned against a set of in-house reference standards.

### **CALCULATION OF RESULTS**

If a microplate spectrophotometer with built-in data calculation program is used refer to the manual for the spectrophotometer and create a program using the concentration stated on the label of each of the CYFRA 21-1 calibrators.

For automatic calculation of CYFRA 21-1 results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator A should be included in the curve with the value 0 ng/mL.
- Interpolation with point-to-point evaluation. Calibrator A should be included in the curve with the value 0 ng/mL.
- Quadratic curve fit method. Calibrator A should be included in the curve with the value 0 ng/mL.

**Note:** 4-Parametric or Linear regression evaluation methods should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CYFRA 21-1 calibrator against the corresponding CYFRA 21-1 concentration (in ng/mL).

The unknown CYFRA 21-1 concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

### Calculation of results with diluted samples

Samples with CYFRA 21-1 concentrations above the measuring range can be diluted with CYFRA 21-1 Calibrator A. The recommended dilution is 1/2.

- 1/2 dilution = 100 µL of specimen + 100 µL of CYFRA 21-1 Calibrator A

The CYFRA 21-1 concentration of the diluted sample is then calculated as:

- Dilution 1/2 : 2 x measured value

### REFERENCES

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9. Stieber P, Hasholzner U, Bodenmüller H, Nagel D, Sunder-Plassmann L, Dienemann H, Meier W, Fateh-Moghadam A. CYFRA 21-1. A new marker in lung cancer. *Cancer* 1993;72(3):707-13.

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# Protocol Sheet

## CYFRA 21-1 EIA REF 211-85

Prepare the components directly before use. Use wash and incubation conditions according to the Instructions.

Step	Vial/Plate	Procedure																																							
1. Prepare CYFRA 21-1 Calibrators	<span style="border: 1px solid black; padding: 2px;">CAL</span> <span style="border: 1px solid black; padding: 2px;">CYFRA 21-1</span> B, C, D, E, F	Add 1 mL of distilled or deionised water to each vial. Allow to stand for at least 15 minutes and mix gently. NOTE: The exact concentration of each calibrator is stated on the label. Do not Vortex.																																							
Prepare CYFRA 21-1 Controls	<span style="border: 1px solid black; padding: 2px;">CONTROL</span> <span style="border: 1px solid black; padding: 2px;">CYFRA 21-1</span> 1, 2																																								
Prepare Wash Solution	<span style="border: 1px solid black; padding: 2px;">WASHBUF</span> <span style="border: 1px solid black; padding: 2px;">25X</span>																																								
Prepare Antibody Solution	<span style="border: 1px solid black; padding: 2px;">CONJ</span> <span style="border: 1px solid black; padding: 2px;">Anti-CYFRA 21-1</span> <span style="border: 1px solid black; padding: 2px;">BIOTIN</span> <span style="border: 1px solid black; padding: 2px;">Anti-CYFRA 21-1</span>																																								
		<table border="1"> <thead> <tr> <th>No. of Strips</th> <th>Tracer, HRP Anti-CYFRA 21-1 (µL)</th> <th>Biotin Anti-CYFRA 21-1 (mL)</th> </tr> </thead> <tbody> <tr><td>1</td><td>50</td><td>1</td></tr> <tr><td>2</td><td>100</td><td>2</td></tr> <tr><td>3</td><td>150</td><td>3</td></tr> <tr><td>4</td><td>200</td><td>4</td></tr> <tr><td>5</td><td>250</td><td>5</td></tr> <tr><td>6</td><td>300</td><td>6</td></tr> <tr><td>7</td><td>350</td><td>7</td></tr> <tr><td>8</td><td>400</td><td>8</td></tr> <tr><td>9</td><td>450</td><td>9</td></tr> <tr><td>10</td><td>500</td><td>10</td></tr> <tr><td>11</td><td>550</td><td>11</td></tr> <tr><td>12</td><td>600</td><td>12</td></tr> </tbody> </table>	No. of Strips	Tracer, HRP Anti-CYFRA 21-1 (µL)	Biotin Anti-CYFRA 21-1 (mL)	1	50	1	2	100	2	3	150	3	4	200	4	5	250	5	6	300	6	7	350	7	8	400	8	9	450	9	10	500	10	11	550	11	12	600	12
No. of Strips	Tracer, HRP Anti-CYFRA 21-1 (µL)	Biotin Anti-CYFRA 21-1 (mL)																																							
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11	550	11																																							
12	600	12																																							
2. Wash	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	Wash each well once with Wash Solution. Use manual or automatic washer.																																							
3. Add calibrators, controls and samples	<span style="border: 1px solid black; padding: 2px;">CAL</span> <span style="border: 1px solid black; padding: 2px;">CYFRA 21-1</span> A, B, C, D, E, F <span style="border: 1px solid black; padding: 2px;">CONTROL</span> <span style="border: 1px solid black; padding: 2px;">CYFRA 21-1</span> 1, 2	50 µL in each well. Mix samples thoroughly by gentle inversion. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 seconds.																																							
4. Add Antibody Solution	<b>ANTIBODY SOLUTION</b>	100 µL in each well																																							
5. Incubate	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	1 hour shaking at 20-25°C																																							
6. Wash	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	Wash each well six times with Wash Solution Use manual or automatic washer.																																							
7. Add TMB HRP-Substrate	<span style="border: 1px solid black; padding: 2px;">SUBS</span> <span style="border: 1px solid black; padding: 2px;">TMB</span>	100 µL in each well																																							
8. Incubate	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	30 min shaking at 20-25°C																																							
9. Read absorbance	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	620 nm																																							
Alt.9 Add Stop Solution	<span style="border: 1px solid black; padding: 2px;">STOP</span>	100 µL in each well																																							
Alt.10 Mix	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	Allow to mix at 20-25°C																																							
Alt.11 Read absorbance	<span style="border: 1px solid black; padding: 2px;">MICROPLA</span>	Read at 405 nm within 15 min																																							



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