Antimicrobial Susceptibility Test:
Microbial Viability Assay Kit-WST

Product Description

Microbial Viability Assay Kit-WST is utilized for bacteria and Fungi viability determination. This kit contains WST-8 and electron mediator. WST-8* (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) produces a water-soluble formazan dye upon bioreduction in the presence of an electron mediator. Since the assay solution is stable in a culture medium, it can be added directly to microbial cell culture and incubated for 1-2 hours or overnight if necessary. The microbial cell viability can be determined at 450 nm absorbance.

*WST-8 patent No. 2,251,850(Canada), 6,063,587(US), 0908453(EP), 2757348(JP)

Product Information

Microbial Viability Assay Kit-WST

Product code	Unit	Components
M439-10	500 tests	WST solution : 1 ml x 5 tubes Electron mediator reagent / DMSO : 0.5ml x 1 tube

One test corresponds to one well of the 96-well plate.

Applications: Bacterial and Fungal Viability Monitoring

Required Materials

Devices, tools

- Microplate Reader with a 450 - 490 nm filter and 650 nm filter (reference)
- 96 well microplate, sterilized clear plate for cell assay
- Multi-pipette (8 channel: 10-200 μl)
- Incubator
- Biological safety cabinet
- Spectrophotometer
- Centrifuge rotor

Reagents

- Microplate Viability Assay Kit-WST [product code: M439]
- Culture media
- Material to be tested
- PBS or other buffers for the preparation of material solutions if cell culture medium cannot be used.

Preparation

Microbial Viability Assay Kit-WST

This kit consists of two solutions, one is WST Solution and the other is Electoron Mediator Reagent.
To prepare a coloring reagent, Mix 9 parts of WST solution and 1 part of Electron Mediator Reagent.

⚠ For Gram-positive bacteria, Fungi, and Vibrio parahaemolyticus assay, please make 8-fold dilution of Electron mediator solution with DMSO or sterile water and prepare the coloring reagent.

⚠ Coloring reagent is stable when it is stored at 4 °C for 1 month. For a long term storage over a year, store at -20 °C.

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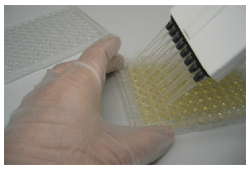
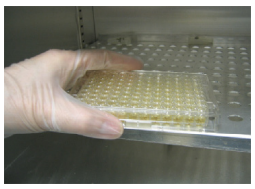
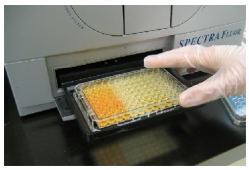
Assay Conditions

When using Microbial Viability Assay Kit-WST for the determination of MIC(Minimum Inhibitory Concentration) of organisms, it is desirable to start with a set number of microbial cells, to develop sufficient color in 2 hours. Below, the method and conditions for using Microbial Viability Assay Kit-WST are described.

Procedure	Precautions and Tips
Culture the microbial cells to be assayed using suitable medium.	
Adjust the absorbance at 550 nm to 0.125 of microbial cell suspension with sterile saline and make an additional 10-fold diluted cell suspension of it with sterile saline. Then, the number of microbial cell is approximately 10^7cfu/ml. (cfu:Colony forming unit)	
Prepare the various concentrations of antibiotic in Mueller -Hinton broth. (e.g. 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03 μg/ml)	
Add 180 μl antibiotic in Mueller-Hinton broth to each well. 	
Inoculate 10 μl organism suspension to each well (final cell density: 10^4 cfu/ml). Make a well of only media to measure background. Add 190 μl of media.	
Incubate the plate for 6 hours at suitable temperature for organisms. 	
Add 10 μl of coloring reagent to each well on the 96 well microplate.	
Place in an incubator for 2 hours to react.	
Take a colorimetric reading on a microplate reader. filter: 450 - 490 nm filter: 650 nm (for background measurement) 	Since bubbles can cause error, make sure there are no bubbles in each well. If there are bubbles, use a pipette tip to remove or use a toothpick to pop them.

Experimental Example

Antimicrobial Susceptibility Test of *Staphylococcus aureus* to Oxacillin

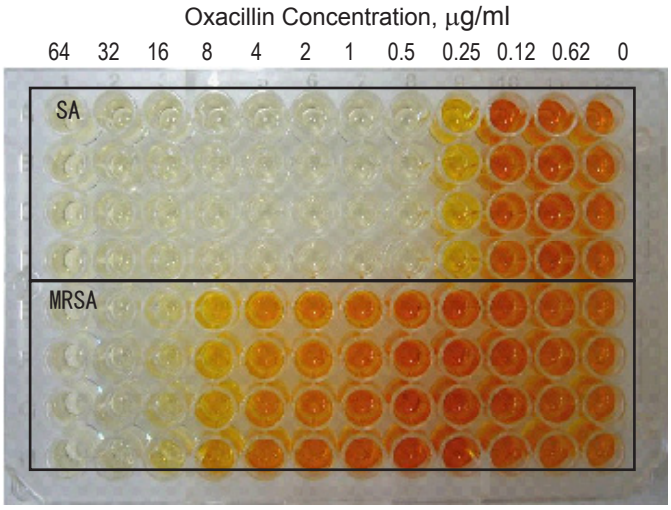
Conditions

Organisms
Methicilin-resistant *Staphylococcus aureus subsp. aureus* (MRSA)
Staphylococcus aureus (SA)

Antimicrobial agent
Oxacillin (beta-lactam antibiotic of the penicillin class)

Procedure

1. Culture MRSA and SA with Mueller-Hinton broth containing various concentrations of Oxacilin for 6 hours at 35°C.
2. Add coloring solution (Microbial Viability Assay Kit-WST) equal to 1/20 volume of the culture medium.
3. Incubate for 2 hours at 35°C.
4. Measure the O.D. at 450 nm to determine the MIC (Minimum Inhibitory Concentration).



The data indicates that MRSA has lower susceptibility than SA.
The MICs of MRSA and SA can be determined as 32 μg/ml and 0.5 μg/ml, respectively. They are close to the MICs determined by CLSI (Clinical Laboratory Standards Institute) method.

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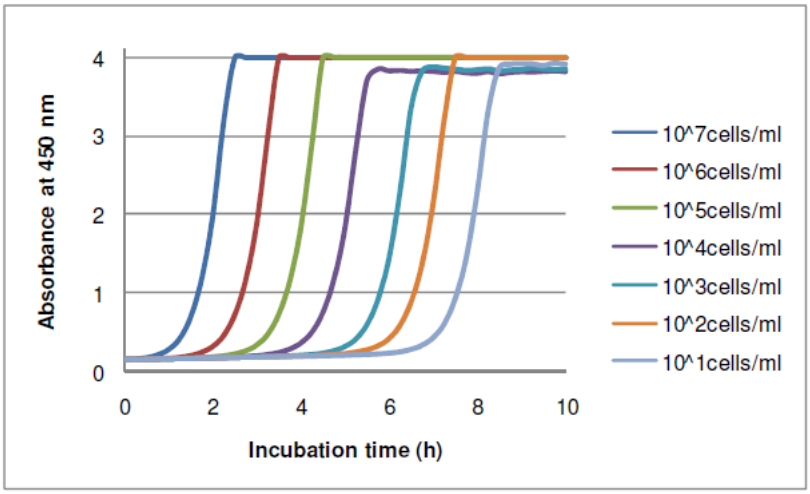
Proliferation Assay (Initial Number of Organisms):
Microbial Viability Assay Kit-WST

Experimental Example

Number of viable organisms can be calculated by following procedure.

Step 1

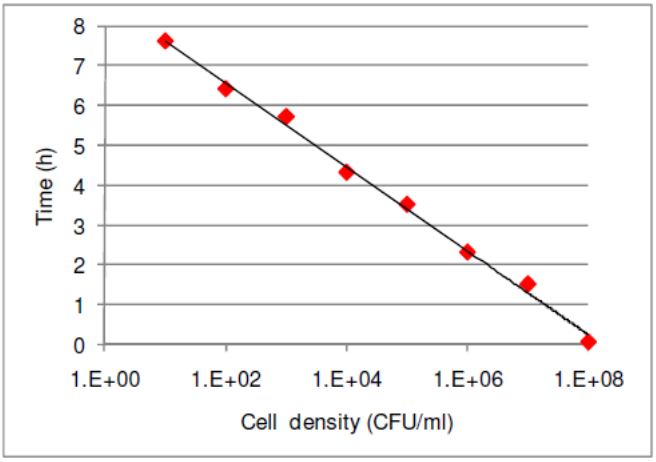
1. Culture microbial cell using suitable culture media.
2. Make serial dilutions of organism suspension (e.g. 10^7, 10^6, 10^5,10^4,10^3, 10^2...0 cfu/ml) and then add dilutions to each well in a 96 well plate.
3. Add Coloring solution with 1/20 volume of the culture medium.
4. Incubate the plate and measure the O.D. every 10 to 15 min at 450 nm.



Step 2

1. Make a calibration curve as following*

   *Each plot represents the time necessary for each cell density to reach 0.5 O.D.



2. Initial number of organisms can be estimated by using calibration curve.

Troubleshooting

Problem	Possible Cause	Solution
The absorbance reading exceeds the upper limit of the machine.	Too many cells per well.	The number of viable microorganisms may increase during the pre-incubation. Prepare a microplate with lower number of organisms for the assay.
	Too long of a incubation time.	Shorten the incubation time.
The color development occurs even though cells are clearly dead when using the kit for cytotoxicity assays.	WST-8 is reduced by the test substance or materials which are generated in the culture media during the assay.	Mix Microbial Viability Assay Kit-WST solution with the substance to test whether the substance reacts or not. If there is a coloration, follow the following step: 1) Before adding the Microbial Viability Assay Kit-WST solution, centrifuge the plate and replace the culture media to remove the test substance or materials in the culture media.
There is high variation in the data.	The assay condition of outer-most wells has changed due to the edge effect.	Do not use the outer-most wells for the assay. Just add media to the outer-most wells.
	Microbial Viability Assay Kit solutions has not been mixed well with the media.	Lightly tap the outside of the well in order to get thel Microbial Viability Assay Kit-WST that is on the well wall to fall into the media.
	There are bubbles on the surface of the media.	Remove the bubbles using a pipet tip or a toothpick.
No color or less color development even though the number of microbial cells seems to have increased.	Cell viability of each microbial cell is low because of high population of cells per well.	Reduce the number of cells for the assay or shorten the incubation time.

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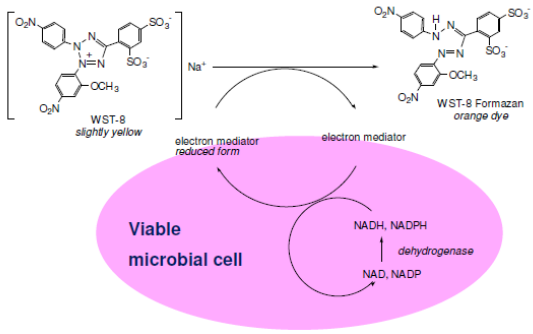
Q&A

Questions concerning the reagent

Q: *What causes color development according to the viable microbila cell number in Microbial Viability Assay Kit-WST?*
A: WST-8 is reduced to an orange-colored formazan through electron mediator by NADH and NADPH activity which are generated by cellular activities. The amount of WST-8 formazen is dependent on the activity of cellular dehydrogenase, so WST-8/ electron mediator system can be used to determine the number of viable microbial cells or microbial cell viabilities.



Q: *Do WST-8 and electron mediator molecules enter into the cell?*
A: There is no clear evidence that these molecules do or do not enter the cell. Generally, a neutrally or positively charged organic molecule such as MTT can enter the cell. Therefore, it is estimated from their charges that electron mediator can enter the cell because it does not have a negative charge, but WST-8 cannot. It is speculated that electron mediator receives an electron from NADH or NADPH at the membrane or inside of the cell and passes the electron to the WST-8 that is around the outer cell membrane.

Q: *What is the stability of the Microbial Viability Assay Kit-WST?*
A: The Microbial Viability Assay Kit-WST is stable for over 1 month at ambient temperature. Therefore, it is possible to ship this kit without dry ice or blue ice. The kit is stable for over one year when stored in a refrigerator.

Q: *What is the toxicity of Microbial Viability Assay Kit-WST?*
A: If you follow the protocol on the technical manual, the cytotoxicity of the assay kit is very low. The kit gives no damage to the microbial cells during the assay.

Questions regarding microbial cells and culture

Q: *What type of culture medium can be used?*
A: Generally, most of the culture medium for organisms culture can be used for the assay without changing the medium.

Q: *How long of a pre-incubation time is required prior to assay?*
A: Pre-incubation is generally not necessary.

Q: *When using Microbial Viability Assay Kit-WST, what number of microbial cell is appropriate?*
A: The appropriate number of cell depends on the type of cell and the type of experiment. The amount of coloration will differ dependent on organism type, even if the cell number per well and coloration times are the same.

Q&A

Q: *Is it possible to do the assay in a 24 or 12 well plate? If so, how much Microbila Viability Assay Kit-WST solution should be used?*
A: Yes, it is possible to assay using plates other than a 96 well plate. Please add Microbial Viability Assay Kit-WST solution with 1/20 volume of the media (if the media is 1 ml, add 50 ul of solution)

Q: *How long of an incubation time is sufficient for the color development?*
A: For antimicrobial susceptibility test, the incubation time is 2 hrs. However, the absorbance will differ between cell types even if the number of cells per well and coloration time are the same. Set an appropriate incubation time to give a proportional relationship between the cell number and the absorbance.

Q: *Are there any materials that can affect the color development?*
A: Reducing agents and materials with reducing activity may react with WST-8 and give a false reading. If the material is considered to have a reducing activity, mix the material solution with Microbial Viability Assay Kit-WST solution and incubate to check whether the material reacts with WST-8 or not. Then, if the material reacts with WST-8, remove the culture medium containing such material from Microbial cells and add new culture medium prior to adding Microbial Viability Assay Kit-WST solution. Dye materials with absorbance around 450-490 nm may affect the reading. However, absorbance from such dyes can be subtracted as a blank.

Q: *How do you subtract the background caused by microbial cells?*
A: Measure the absorbance at 600-650 nm of the well as a reference. Then, the absorbance at 600-650 nm is subtracted from the absorbance of the same well measured at 450 nm to eliminate the background that comes from turbidity.

Q: *The cell culture in the well contains material which has an absorbance around 450 nm.*
A: Use a couple of wells for a background absorbance measurement to subtract the total absorbance of the sample wells. Prepare the well for background measurement which contains all materials except for cells. Measure the background absorbance of the well at 450 nm, and then subtract the background absorbance from the absorbance of the sample well containing all materials and cells.
note: If the background absorbance from the material is too high to subtract, remove the culture medium and wash cells with fresh media and add the same volume of fresh media to the well prior to adding the Microbial Viability Assay Kit-WST solution.

Q: *What should be done regarding materials which may increase the color development and interfere with the Microbial Viability Assay?*
A: Determine whether the material interferes with the assay by adding the Microbial Viability Assay Kit-WST to the material which may interfere with the assay and incubate for a general assay period.
a)If there is no color development during the incubation, the material does not react with the Microbial Viability Assay Kit-WST solution.
b)If there is color development during the incubation, the material does react with the Microbial Viability Assay Kit-WST. Prepare a couple of wells for a background absorbance measurement which contains all material except for cells. Measure the absorbance of the background well at 450 nm and subtract this background from the absorbance of the wells containing all materials and cells.
note: If the color development is too high to subtract, remove the culture media, wash the cells with fresh media, and then add the same volume of fresh media to the well prior to adding the Microbial Viability Assay Kit-WST solution.

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Microbial Viability Assay Kit-WST

Applicable Organisms

Bacteria
Acetobacter Pasteurianus
Acinetobacter baumannii
Aerococcus viridans
Aeromonas hydrophila subsp. hydrophila
Alcaligenes faecalis subsp. faecalis
Arthrobacter aurescens
Bacillus cereus
Bacillus circulans
Bacillus coagulans
Bacillus subtilis subsp. subtilis
Bacillus thuringiensis
Bordetella bronchiseptica
Brevibacillus brevis
Cedecea davisae
Chromobacterium violaceum
Citrobacter freundii
Corynebacterium diphtheriae
Corynebacterium glutamicum
Cronobacter sakazakii
Edwardsiella tarda
Empedobacter brevis
Enterobacter aerogenes
Enterobacter cloacae
Enterobacter cloacae subsp. cloacae
Enterococcus casseliflvus
Enterococcus faecalis
Enterococcus faecium
Enterococcus gallinarum
Enterococcus hirae
Erwinia persicina
Escherichia coli
Haemophilus influenzae
Hafnia alvei
Klebsiella pneumoniae
Lactobacillus brevis
Lactobacillus casei
Lactobacillus delbrueckii subsp. lactis
Lactobacillus plantarum
Lactobacillus plantarum subsp. plantarum
Lactobacillus rhamnosus
Lactobacillus sakei subsp. sakei

Bacteria
Lactococcus lactis supsp. cremoris
Lactococcus lactis subsp. lactis
Leuconostoc mesenteroides subsp. mesenteroides
Listeria innocua
Listeria monocytogenes
Microbacterium laevaniformans
Micrococcus luteus
Morganella morganii subsp. morganii
Nocardia asteroides
Pasteurella pneumotropica
Pediococcus pentosaceus *
Providencia alcalifaciens
Proteus mirabilis
Pseudomonas aeruginosa
Pseudomonas fluorescens
Rhodococcus rhodochrous
Salmonella enterica subsp. enterica
Serratia marcescens
Sporosarcina ureae
Staphylococcus aureus subsp. aureus
Staphylococcus epidermidis
*Streptococcus gordonii**
*Streptococcus mitis**
Streptococcus pyogenes
Vibrio alginolyticus
Vibrio fluvialis
Vibrio parahaemolyticus
Yersinia enterocolitica subsp. enterocolitica

* Under anaerobic condition

Fungus
Aspergillus oryzae
Candida albicans
Candida guilliermondii
Candida krusei
Candida parapsilosis
Candida tropicalis
Candida utilis
Saccharomyces cerevisiae
Zygosaccharomyces rouxii