
remel

SODIUM CARBONATE

INTENDED USE

Remel Sodium Carbonate is a reagent recommended for use in qualitative procedures with the arylsulfatase test to biochemically differentiate rapid- and slow-growing *Mycobacterium* species.

SUMMARY AND EXPLANATION

Whitehead et al. described a liquid arylsulfatase test with phenolphthalein sulfate as the substrate and an incubation time of 2 weeks.¹ He found it could be used to differentiate virulent tubercle bacilli from nonvirulent strains. Wayne et al. reported all mycobacteria, except *Mycobacterium rhodochrous*, produce the enzyme arylsulfatase.² The differences in enzyme activity were found to be related to differences in growth rate and permeability of the organisms. Wayne et al. formulated liquid and solid arylsulfatase media.³ Kubica and Rigdon used the 3-day arylsulfatase test to differentiate *Mycobacterium fortuitum* complex from saprophytic *Mycobacterium* spp.⁴

PRINCIPLE

The arylsulfatase test measures the ability of certain mycobacteria to produce the enzyme, arylsulfatase, which attacks the substrate phenolphthalein sulfate, releasing free phenolphthalein. This reaction is demonstrated by development of a red color in the medium after addition of sodium carbonate.

REAGENTS (CLASSICAL FORMULA)*

Sodium Carbonate 2N (CAS 497-19-8) 106.0 g
Demineralized Water (CAS 7732-18-5) 1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect product from light.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.^{6,7}

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Dubos Polysorbate Albumin Broth (REF R060854), (7) Pipette, (8) Arylsulfatase Broth 3 Day (REF R060192) or 2 Week (REF R060196), or Arylsulfatase Agar (REF R08026).

PROCEDURE

Follow established laboratory safety procedures when working with acid-fast cultures and specimens. Consult appropriate references when necessary for detailed procedural information on specimen processing, media inoculation, and identification of test isolates.^{6,7}

1. Prepare a barely turbid suspension of the test isolate in Dubos Polysorbate Albumin Broth.
2. Incubate aerobically at 35-37°C for 7 days.
3. Inoculate 1 drop of the broth suspension into a tube of Arylsulfatase 3-Day Broth, Arylsulfatase 2-Week Broth, or Arylsulfatase Agar.
4. Incubate the 3-day agar or broth test for 72 hours and the 2-week broth test for 14 days at 35-37°C.
5. After incubation, add six drops of Sodium Carbonate to each tube of broth or 1 ml to the agar butt.
6. Observe for a pink-red color development. Refer to the color chart in the CDC manual for color grading.⁵ The color usually develops immediately, but in the case of low enzyme concentration longer incubation after addition of Sodium Carbonate may be required. Wait 30 minutes before recording the result as negative.³

INTERPRETATION**Arylsulfatase Broth:**

Positive Test - Pink-red color development

Negative Test - No color change

Arylsulfatase Agar:

Positive Test - Pink-red band on the surface of the agar within 30 minutes

Negative Test - No color change within 30 minutes

QUALITY CONTROL

All lot numbers of Sodium Carbonate have been tested using the following quality control organisms and have been found to be acceptable. Testing of positive and negative control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Mycobacterium fortuitum</i> ATCC® 6841	CO ₂ , 3 days or 2 weeks @ 35-37°C	Positive
<i>Mycobacterium tuberculosis</i> ATCC® 25177	CO ₂ , 3 days or 2 weeks @ 35-37°C	Negative

LIMITATIONS

1. Because many species of *Mycobacterium* produce arylsulfatase, differentiation within the genus using this test requires control of substrate concentration, inoculum size, and incubation time. Even those strains with low enzyme activity will produce a positive arylsulfatase test if a large inoculum is used with a high substrate concentration and incubated for an extended period of time.⁸
2. The development of any pink color within 30 minutes after the addition of Sodium Carbonate is considered a positive test.³
3. An occasional strain of *Mycobacterium kansasii* may produce a positive 3-day test. Such isolates can be differentiated from *M. tuberculosis* by growth rate and pigment.⁸
4. Test a few uninoculated tubes of each lot number of Arylsulfatase Broth or Agar for free phenolphthalein by adding a few drops of Sodium Carbonate. Formation of a pink color indicates premature breakdown of either the medium or the powdered phenolphthalein disulfate salt.⁵




BIBLIOGRAPHY

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PACKAGING

REF R21267, Sodium Carbonate.....25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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