



## PRODUCT SPECIFICATION SHEET

### Arret & Kirshbaum Medium/Sporulating Agar/ A.K. Agar No.2 (DM319)

#### Intended Use

Recommended for production of spores of *Bacillus subtilis* ATCC 6633

#### Product Summary and Explanation

Sporulation is a developmental cycle that involves a complex network of gene expressions that results in the formation of spores. Sporulation is a strategy used by many organisms to survive in conditions that are too harsh for vegetative growth. Spores are resistant to environmental factors and can survive without water, at extreme temperatures and pressure. Many organisms use sporulation, including bacteria, fungi, protozoa, algae, and ferns. During sporulation, a vegetative cell undergoes a developmental change to form a metabolically inactive endospore. Arret & Kirshbaum Medium is formulated by Arret and Kirshbaum used for the production of spores of *Bacillus subtilis* ATCC 6633, which is used in the Penicillin Milk Test procedure.

#### Principles of the Procedure

This medium is highly nutritious due to the presence of yeast extract, beef extract, pancreatic digest of gelatin and Tryptone which also serve as a source of vitamins and essential amino acids. Dextrose acts as the source of energy as well as the fermentable carbohydrate. Manganous sulphate stimulates the sporulation process. A fresh slant culture of *Bacillus subtilis* is washed with sterile physiological saline onto the surface of Roux bottles containing 300 ml sterile medium. The bottles are incubated at 35°C for 5 days and the growth is harvested with 50 ml of sterile physiological saline which is separated by centrifuging the suspension. The sediment is re-suspended in fresh sterile saline and heated at 70°C for 30 minutes to kill vegetative cells and obtain the spore suspension. This spore suspension can be stored for months for use in detection of penicillin/ antibiotic residues in milk and dairy products (2)

#### Formula / Liter

Ingredients	g / L
Gelatin Peptone	6.00
Tryptone	4.00
Yeast extract	3.00
Beef extract	1.50
Dextrose	1.00
Manganous sulphate	0.30
Agar	15.00
Final pH 6.6±0.2 (at 25°C)	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

#### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating eyes, respiratory system, and skin.

#### Directions

1. Suspend 30.8 grams of the medium in one liter of distilled water.
2. Heat to boiling, if necessary, to dissolve the medium completely.
3. Dispense 300 ml amounts in Roux or other suitable bottles.
4. Sterilize by autoclaving at 121°C for 15 minutes. / Validated cycle.

**Advice:** Do not autoclave till the medium has been completely dissolved.





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## Quality Control Specifications

Dehydrated Appearance	Light yellow coloured free flowing powder
Prepared Medium	Lightly amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 3.08% Solution	pH: 6.6±0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

**Expected Cultural Response:** Cultural characteristics observed after an incubation of 30-35°C for 5 days.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Sporulation	Recovery
1.	<i>Bacillus subtilis</i> ATCC 6633	50 -100	luxuriant	positive	>=70%
2.	<i>Bacillus megaterium</i> ATCC 9855	50 -100	luxuriant	positive	>=70%

## Test Procedure

Refer to appropriate references for instructions on specific material being tested.

## Results

Refer to appropriate references and standard procedures for interpretation of results.

## Storage

Store the sealed bottle containing the dehydrated medium 10-30°C and prepared medium at 20-25°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

## Expiration

1. Refer to the expiration date stamped on the container.
2. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color.
3. Expiry applies to the medium in its intact container when stored as directed.
4. Product performance is better if used within the mentioned expiry period.

## Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.
3. Product performance is better if used within the stated expiry period.

## Packaging

**Product Name:** Arret & Kirshbaum Medium

**Product Code:** DM319

**Available Pack sizes:** 100g,500g

## References

1. Arret and Kirshbaum, 1959, J. Milk and Food Tech., 22:329.
2. Richardson (Ed.), 1995, Standard Methods for the Examination of Dairy Products, 15th Ed., APHA, Washington D.C.

## Further Information

For further information please contact your local MICROMASTER Representative.





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