MICROGEN
BIOPRODUCTS

Microgen™ Bacillus-ID

For the identification of mesophilic *Bacillus spp* from food and food ingredient samples

Instructions for Use

REF **MID-66**

MICROGEN Bacillus-ID

Quick Reference

STEP 1	**SELECT SUFFICIENT COLONIES TO ACHIEVE THE DESIRED INOCULUM DENSITY**
STEP 2	**EMULSIFY IN BACILLUS SUSPENDING BROTH**
STEP 3	**TRANSFER 4 DROPS TO EACH MICROWELL**
STEP 4	**INCUBATE 30°C FOR 24 HOURS**
STEP 5	**READ AND RECORD RESULTS AT 24 HOURS RETURN STRIPS TO 30°C INCUBATOR**
STEP 6	**READ, ADD REAGENTS AND RECORD RESULTS AT 48 HOURS**
STEP 7	**INTERPRET USING MICROGEN IDENTIFICATION SYSTEM SOFTWARE**

The Microgen Bacillus-ID system is intended to be used for the identification of mesophilic *Bacillus spp*. isolated from samples of food and food ingredients.

The following species can be identified using the Microgen Bacillus-ID system.

***Bacillus* species**	*B. pumilus*
B. cereus group	*B. licheniformis*
B. firmus	*B. megaterium*
B. badius	
B. laevolacticus	***Vergibacillus* species**
B. coagulans	*V. pantothenticus*
B. lentus	
B. amyloliquefaciens	***Paenibacillus* species**
B. subtilis	*P.alvei*
B. circulans	*P. polymyxa*
B. insolitus	*P. macerans*
B. thiaminolyticus	
B. freudenreichii	***Brevibacillus* species**
B. globisporus	*Br. brevis*
B. sphaericus	*Br. laterosporus*

Note: *B.cereus group consists of B. cereus, B. thuringiensis and B. mycoides* and *B. weihenstephanensis.* On the basis of routinely employed biochemical tests, these species are indistinguishable. (See Limitations)

PRINCIPLE

The Microgen Bacillus-ID identification system consists of 2 microwell strips (labelled BAC 1 and BAC 2), each containing 12 dehydrated substrates for the performance of either carbohydrate fermentation tests or other biochemical based tests. The last well in the second strip is a carbohydrate fermentation control well for use as a reference well in the interpretation of these tests. The selection of the substrates included in the test panel has been determined using computer based analysis of all available substrates for the identification or differentiation of this group of organisms (1).

Identification of isolates is achieved by recording the results visualised by a colour change after 24 and 48 hours hours incubation at 30°C and the addition of appropriate reagents (Indole, Nitrate and VP tests) after 48 hours.

These results are then analysed using the Microgen Identification System Software (MID-60)

Each Microgen Bacillus-ID test consists of the following 24 biochemical reactions:

Well	Substrate	Reaction	Positive	Negative
1	Arabinose	Fermentation of specific sugars producing acid end products changes the Phenol Red indicator from red to yellow	Yellow	Red
2	Cellobiose			
3	Inositol			
4	Mannitol			
5	Mannose			
6	Raffinose			
7	Rhamnose			
8	Salicin			
9	Sorbitol			
10	Sucrose			
11	Trehalose			
12	Xylose	Fermentation of specific sugars producing acid end products changes the Phenol Red indicator from red to yellow	Yellow	Red
13	Adonitol			
14	Galactose			
15	Methyl-D-Mannoside			
16	Methyl-D-Glucoside			
17	Inulin			
18	Melezitose			
19	Indole	Indole is produced from tryptophan and gives a pink/red complex when Kovac's reagent is added.	Pink / Red	Colourless / Yellow
20	ONPG	Hydrolysis - ONPG hydrolysis by B-galactosidase results in the production of yellow ortho-nitrophenol.	Yellow	Colourless
20 Plus reag	Nitrate	Nitrate is reduced to nitrite which forms a deep red complex after the addition of α-Naphthylamine and Sulphanilic Acid	Red	Colourless
21	Arginine Dihydrolase	Arginine is converted to ornithine, ammonia and CO_2 by arginine dihydrolase resulting in an increase in pH and a change in colour of the bromothymol blue from green to blue. At 48 hours green reactions are negative.	Green/ Blue Blue	Yellow Yellow / Green
22	Citrate Utilisation	Utilisation of citrate (only carbon source) leading to a pH increase giving a colour change in bromothymol blue from yellowy green to blue.	Blue	Yellow / Light Green
23	Voges Proskauer	Acetoin production from glucose is detected by the formation of a pink / red complex after the addition of alpha naphthol and creatine in the presence of KOH.	Pink / Red	Straw colour
24	Control	Carbohydrate Control	Red	Red

REAGENTS

Kit Contents (20 tests)

Holding frame for test microwell strips
Result forms
Instructions for use
20 MID66c microwell strips (BAC1 and BAC2) in individual foil pouches
20 MID66b Bacillus Suspending Medium

Additional Materials Required (not supplied in the kit)

Microgen Identification System Software (MID-60) requires version 1.1.16.19 onwards
Mineral Oil
VP I and VP II Reagents
Nitrate A&B Reagents
Kovac's Reagent
Colour chart for reading results – A4 size available from your distributor on request.
Blood agar / Nutrient agar plates
Sterile bacteriological loops
Sterile plain swabs
Sterile Pasteur pipettes
Incubator (30°C), not fan assisted
Refrigerator (2 - 8°C)
Marking Pen
Gram stain reagents
Catalase test reagents
Microscope
Microscope slides
Vortex mixer

STORAGE

The microwell strips are stable in the unopened foil pouches at 2 - 8°C until the expiry date stated. The Bacillus suspending broth should be stored at 2 – 8°C.

INSTRUCTIONS FOR USE
(Before using this product, refer to Precautions and Limitations)

1. Selection of colonies for identification

1.1. Isolates must be tested from a pure culture on non selective media eg Blood Agar. Subculturing from a primary plate will be required.

1.2. Prior to inoculation into the Microgen Bacillus ID, isolates should be checked to ensure they are members of the genus *Bacillus*

1.2.1. Gram positive bacillus,
1.2.2. Endospore forming
1.2.3. Catalase positive
1.2.4. Optimal growth temperature between 25 and 45°C i.e. Mesophilic. Isolates growing at < 25°C (Psychrophiles) or isolates growing at >45°C (Thermophiles) are not identified by this product.

2. Inoculum preparation

2.1. Bring the suspending broth and microwell strips to room temperature before inoculation.

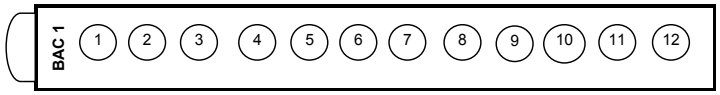
2.2. Remove colonies from an 18-24 hour pure culture using a sterile loop or swab and emulsify it in a vial of Bacillus Suspending medium (5ml). Several sweeps with the swab may be required.

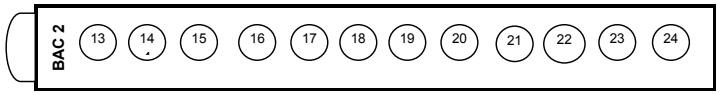
2.3. Mix thoroughly eg using a vortex mixer suspension equivalent to a MacFarland 2.0 standard and allow particulates to settle prior to inoculating the strips. More than one plate of pure culture may be required to achieve this.

2.4. Inoculate the strips within 10 minutes of preparing and mixing the suspension.

3. Inoculation and Incubation

3.1 Remove the microwell strips from the foil pouch and place in the holding tray.





3.2 Carefully peel back the adhesive strips sealing the microwells. **Do NOT discard the sealing strips as they will be required later.**

3.3 Using a sterile Pasteur pipette transfer 4 drops (100-125µl) of the bacterial suspension to each well of both microwell strips.

3.4 After inoculation, overlay well 21 (arginine) with 3-4 drops of mineral oil**.**

3.5 Seal the top of the microwells with the adhesive strips peeled back earlier and incubate at 30°C for 24 hours and 48 hours**. Ensure that the punctures in the adhesive strip are positioned above the citrate and ONPG microwells, on the BAC 2 strip, and that a good seal is achieved.**

3.6 As a purity check, transfer 1 drop of the organism suspension onto an appropriate non selective agar plate. Incubate the plate aerobically at 30°C for 18 - 24 hours.

4. Interpretation

4.1 After 24 hours peel back the adhesive strip and record all positive results for wells 1 to 18 (carbohydrates) with reference to the control well. Anything more orange or yellow in colour compared to the control well should be scored as positive. The arginine, ONPG and citrate results should be read against the colour chart and recorded. Record the results on the forms provided, reseal the adhesive strips and return them to 30°C for a further 24 hours.

4.2 After 48 hours incubation add the appropriate reagents to the following microwells in the second microwell strip:

4.2.1 Add 2 drops of Kovac's reagent to well 19. Read and record the results after 60 seconds. Formation of a pink/red colour indicates a positive result.

4.2.2 Add 1 drop each of the VP I and VP II reagents to well 23 and read after 15-30 minutes. Formation of a pink/red colour indicates a positive result.

4.2.3 Perform the nitrate reduction test on well 20 after reading and recording the ONPG result. Add 1 drop each of Nitrate A and Nitrate B reagents to the well and read after 60 seconds.

4.3. Record these additional results on the forms provided.

4.4. Report Form

MICROGEN BACILLUS-ID 24 TEST

REPORT FORM

MICROGEN BIOPRODUCTS

Lab. No. F4560

Specimen Type Cooked Rice

Date 12-10-04

	Bacillus Strip 1 (BAC 1)												Bacillus Strip 2 (BAC 2)												
Well Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	20	24
Reaction	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONPG	ARG	CIT	VP	NIT	NEG
24 hours	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−		−	+	−			
48 hours	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−	+	+	
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	
Sum of Positive Reactions	0			0			2			6			0			0			1			3			

Profile No: 00260013 Final Identification: Bacillus cereus group

PRECAUTIONS

1. The Microgen Bacillus-ID system is intended for use by qualified laboratory personnel using aseptic technique, appropriate microbiological precautions and after reading these Instructions For Use.

2. Used materials must be disposed of safely by autoclaving, incineration or immersion into an appropriate disinfectant prior to disposal.

3. The microwell strips **must not** be incubated in a CO_2 or fan forced incubator.

4. Always read carbohydrate fermentation tests after reference to the Control microwell (well 24, strip 2).

5. Carbohydrate fermentation tests should be read after both 24 and 48 hours incubation. If a test is positive after 24 hours incubation but is negative after 48 hours incubation, the positive result should be recorded.

LIMITATIONS

1. The Microgen Bacillus-ID identification system is designed to identify bacteria belonging to the genus ***Bacillus.*** It cannot be used to identify organisms belonging to other genera.

2. On the basis of routinely employed biochemical tests, *B. cereus group consists of B. cereus, B. thuringiensis and B. mycoides* and *B. weihenstephanensis* these species are indistinguishable. The following information may assist further in achieving satisfactory differentiation.

Organism	Motility
B. cereus	+
B. thuringiensis	+
B. mycoides	-
B. weihenstephanensis	?

3. *B. subtilis, B. amyloliquefaciens, B. licheniformis* and *B. pumilus* belong to the *B. subtilis* group. As these species are closely related the performance of some additional tests may be required to achieve satisfactory differentiation.

4. Inoculation of a purity plate from the suspending broth used is recommended as it will confirm that a single species was inoculated into the test strips.

5 The Microgen Bacillus-ID identification system will only identify organisms with an optimal growth temperature between 25 and 45°C i.e. Mesophilic. Isolates growing at < 25°C (Psychrophiles) or isolates growing at >45°C (Thermophiles) are not identified by this product.

QUALITY CONTROL

The performance of the Microgen Bacillus-ID system should be monitored using appropriate control strains. The following are recommended for independent laboratory assessment::

Well Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	20	24
Reaction	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONP	ARG	CIT	VP	NIT	NEG
B. licheniformis ATCC 14580,	-	-	-	+	+	-	-	+	+/-	-	+	+/-	-	-	-	+/-	-	-		+	+	-			
NCTC 10341	-	-	+	+	+	+/-	+/-	+	+	+	+	+	-	+/-	-	+	-	-	-	+	+	+	+	+	
B. cereus ATCC 11778, NCTC	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-		-	+	-			
10320	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	
P. macerans ATCC 8244,	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+/-	+		+	-	-			
NCTC 6355	+	+	+	+	+	+	+	+	+/-	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	
P. alvei ATCC 6344, NCTC	-	+/-	+/-	-	-	+	-	+	-	+	+	-	+	-	-	-	-	-		+	-	-			
6352	-	+/-	+/-	-	-	+	-	+	-	+	+	-	+	+/-	-	+	-	-	+	+	-	-	-	-	

REFERENCES

1. Lapage S.P, S.Bascombe, W.R. Willcox and M.A.Curtis. 1973 Identification of Bacteria by Computer: General Aspects and Perspectives J.Gen. Microbiol. **77**: 273 -290

DATABASE

	TESTS																							
	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONP	ARG	CIT	VP	NIT
P. alvei (2)	0.1	50	52	0.1	35	45	44	21	20	52	43	0.1	98	43	0.1	59	0.1	9	99	65	0.1	0.1	94	1
B. amyloliquefaciens (4)	86	99.9	45	55	79	67	0.1	99	94	99	90	66	0.1	23	0.1	96	40	1	0.1	68	0.1	56	94	74
B. badius	0.1	0.1	1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	99.9	0.1	0.1
B. cereus Group (1)	0.1	67	2	0.1	8	0.1	0.1	73	0.1	54	87	1	0.1	5	0.1	1	0.1	1	0.1	1	62	50	47	64
B. circulans	89	98	36	89	98	96	48	99.9	63	99	99	96	0.1	66	14	51	38	30	0.1	63	1	2	39	17
B. coagulans	52	60	26	34	91	62	39	68	24	86	93	60	6	96	10	57	4	3	0.1	81	0.1	0.1	93	25
B. firmus	6	12	1	74	20	4	0.1	7	7	92	70	3	0.1	6	1	2	4	1	4	20	5	26	75	63
B. freudenreichii	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	33	0.1	99.9
B. globisporus	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	99.9	0.1	0.1	0.1	0.1
B. insolitus	99.9	99.9	50	99.9	0.1	50	0.1	99.9	50	99.9	50	99.9	0.1	50	50	99.9	50	50	0.1	99.9	0.1	99.9	99.9	50
B. laevolacticus	0.1	99.9	0.1	99.9	99.9	99.9	60	99.9	99.9	99.9	99.9	0.1	0.1	99.9	80	99.9	99.9	99.9	0.1	60	20	0.1	99.9	20
B. lentus	57	74	11	65	98	67	50	72	26	89	84	37	2	35	50	26	22	26	17	45	0.1	18	55	8
B. licheniformis (4)	50	50	50	95	98	65	59	98	93	98	98	99	43	50	37	50	83	30	1	90	95	72	92	86
B. megaterium	66	63	57	74	12	75	1	45	51	88	79	63	0.1	63	2	34	50	43	0.1	70	0.1	31	53	19
V. pantothenticus (5)	0.1	94	28	50	99.9	0.1	99.9	99.9	78	89	99.9	0.1	0.1	99.9	0.1	99.9	0.1	0.1	0.1	78	0.1	22	50	50
B. pumilus (4)	93	99	20	99	98	70	10	99	9	99	99	99	30	50	68	44	12	1	1	50	1	60	92	1
B. sphaericus	0.1	0.1	1	1	1	1	0.1	0.1	0.1	1	2	0.1	0.1	4	0.1	0.1	0.1	0.1	0.1	6	1	47	11	14
B. subtilis (4)	95	98	79	96	79	47	51	91	94	99	99	74	1	17	1	93	82	1	0.1	84	0.1	60	99	65
B. thiaminolyticus	0.1	91	91	0.1	99.9	99.9	0.1	99.9	0.1	99.9	99.9	0.1	9	99.9	91	99.9	0.1	99.9	27	99.9	4	99.9	27	18
P. polymyxa (2)	50	50	0.1	50	50	50	17	50	0.1	50	99	95	0.1	95	24	99	74	30	0.1	95	0.1	10	92	72
P. macerans (2)	99	99	25	97	99	97	77	96	45	99.9	99.9	99	2	99.9	54	85	85	78	0.1	95	1	1	64	36
Br. brevis (3)	1	7	7	16	1	1	1	1	1	6	8	2	0.1	5	0.1	1	1	0.1	1	24	6	31	50	53
Br. laterosporus (3)	3	77	3	82	82	0.1	3	88	3	6	99	15	0.1	1	0.1	3	1	0.1	13	3	0.1	31	79	70

Notes:

1. *B.cereus* Group includes *B. cereus*, *B. thuringiensis*, *B. mycoides* and *B. weihenstephanensis*
2. *B. alvei*, *B. polymyxa* and *B. macerans* now *Paenibacillus* spp.
3. *B.brevis* and *B.laterosporus* now *Brevibacillus* spp.
4. *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis* and *B.pumilus* belong to the *B.subtilis* group
5. *B. pantothenicus now Vergibacillus pantothenicus*

Figures denote percentage positive strains

Colour chart/Farbtafel/Tableau de couleurs

Microgen™ Bacillus ID MID-66

Read strips at 24 and 48 hours

WELL/NAPFCHEN /GODET	24	1 to 18	1 to 18	19	20	21	22	23	(20) Plus reagents
Reaction	Carbohydrate Negative Control 24/48 hours	Carbohydrate Fermentation 24 hours (*)	Carbohydrate Fermentation 48 hours (*)	Indole 48 hours	O.N.P.G. 24/48 hours	Arginine 24/48 hours	Citrate 24/48 hours	VP 48 hours	Nitrate 48 hours
Carbohydrate Negative (Well 24 (*))									
Positive									

CAUTION: Keep out of direct sunlight. Due to laminate discolouration and paper ageing, the colours on this chart will change.

These colours are provided as general guide to the range of test colours.

Legend:

- ○ Appropriate reagents to be added **at 48 hours**, prior to reading.
- ◯ Overlaid with sterile mineral oil.

(*) With some Bacillus the negative carbohydrate fermentation control colour may shift from red to orange over time, when scoring a results in wells 1 to 18 they **must** be read with reference to the negative control in well 24

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WF6679/2005/02