

# remel ALN Kit

REF R211356.....25 Disks/Vial

## 1. INTENDED USE

Remel ALN Kit is recommended for use in qualitative procedures for detection of the enzyme, L-alanyl-L-alanylaminopeptidase in bacterial cells. It may be used for rapid differentiation of *Fusobacterium* spp. from *Bacteroides* spp.

## 2. SUMMARY AND EXPLANATION

The extent of anaerobe identification is a concern to laboratories due to the time and expense involved with some methods.<sup>1,2</sup> Anaerobe isolates from sites of serious infections and normally sterile body sites should be definitively characterized. However, presumptive grouping and preliminary identification based on rapid techniques can provide clinically relevant information. Detection of the enzyme L-alanyl-L-alanylaminopeptidase is a rapid, cost-effective procedure for screening cultures by presumptively placing organisms in the appropriate genus (*Bacteroides* spp. are ALN-positive, while *Fusobacterium* spp. are ALN-negative).

## 3. PRINCIPLE

The ALN disk contains a substrate, L-alanyl-L-alanyl- $\beta$ -naphthylamide. This substrate is hydrolyzed by an aminopeptidase, releasing  $\beta$ -naphthylamine. Following the addition of the Cinnamaldehyde Reagent,  $\beta$ -naphthylamine reacts with  $\rho$ -dimethylaminocinnamaldehyde to form a red colored complex, indicating a positive result.

## 4. REAGENTS

Reactive Ingredients:

Disks: L-alanyl-L-alanyl- $\beta$ -naphthylamide

Reagent:  $\rho$ -dimethylaminocinnamaldehyde

## 5. PRECAUTIONS

**CAUTION!** May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact.

### DANGER



H360FD	May damage fertility. May damage the unborn child
P201	Obtain special instructions before use
P202	Do not handle until all safety precautions have been read and understood
P281	Use personal protective equipment as required
P308+P313	IF exposed or concerned: Get medical advice/attention

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. Refer

to Safety Data Sheet for additional information on reagent chemicals.

## 6. 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. Do not freeze or overheat. Allow product to equilibrate to room temperature before use.

A precipitate formed at storage temperatures or a color variation from straw to pink in the Cinnamaldehyde Reagent is common and does not affect performance.

## 7. PRODUCT DETERIORATION

This product should not be used if (1) the disk color has changed from white, (2) the expiration date has passed, or (3) there are other signs of deterioration. Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

## 8. SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.<sup>3,4</sup>

## 9. MATERIALS SUPPLIED

(1) ALN Disks, (2) Cinnamaldehyde Reagent (5 ml).

## 10. 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) Forceps, (7) Microscope slide, (8) Demineralized water, (9) Plastic applicator sticks.

## 11. PROCEDURE

Prior to testing, verify that the test isolate is an anaerobic Gram-negative rod. The ALN test may be performed from a number of commonly used anaerobic media, such as anaerobic blood agar, KV medium, PV medium, and anaerobe PEA.

1. Place an ALN disk on a microscope slide or in a petri dish lid.
2. Moisten the disk with demineralized water taking care to not oversaturate.
3. Inoculate the disk with a visible inoculum removed from a pure, 18-72 hour culture using a sterile loop or plastic applicator stick.
4. Rub the inoculum across the disk (use a visible inoculum).
5. Incubate for two (2) minutes at room temperature.
6. Add one (1) drop of the Cinnamaldehyde Reagent and observe for thirty (30) seconds for a color change to pink or red.

## 12. INTERPRETATION

Positive Test - Red or pink color development

Negative Test - No color development

### 13. EXPECTED VALUES (% POSITIVE)

Anaerobe Isolate	Anaerobe Disk		Additional Test		
	ALN	PRO	PYR	CAT	IND
<i>Bacteroides fragilis</i> group:					
<i>B. caccae</i>	99	0	0	30	0
<i>B. eggerthii</i>	99	0	0	0	99
<i>B. distasonis</i>	99	10	20	90	0
<i>B. fragilis</i>	99	0	25	99	0
<i>B. ovatus</i>	99	0	0	85	95
<i>B. thetaiotaomicron</i>	99	0	0	99	95
<i>B. uniformis</i>	99	0	0	0	95
<i>B. vulgatus</i>	99	0	60	0	0
<i>B. splanchnicus</i> *	99	20	99	0	99
<i>B. ureolyticus</i>	0	0	0	5	0
Other <i>Bacteroides</i> spp.	99	0	V	V	V
<i>Fusobacterium mortiferum</i>	0	0	99	0	0
<i>F. necrophorum</i>	0	0	0	0	99
<i>F. nucleatum</i>	0	0	0	0	99
<i>F. varium</i>	0	0	99	0	70
<i>Capnocytophaga</i> spp.	99	99	0	0	0

PRO = PRO Kit (REF R211357), PYR = PYR Kit (REF R211172); CAT = Catalase; IND = Spot Indole; V = Species variation;

\* = Bile resistant, not *B. fragilis* group (uncommon isolate)

### 14. QUALITY CONTROL

All lot numbers of ALN Kit have been tested using the following quality control organisms and have been found to be acceptable. Testing of 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>Bacteroides fragilis</i> ATCC® 25285	Ambient, 2 min @ 15-30°C	Positive
<i>Fusobacterium nucleatum</i> ATCC® 10953	Ambient, 2 min @ 15-30°C	Negative

### 15. PERFORMANCE CHARACTERISTICS

In a comparative study with another commercially available product, there was 100% correlation between Remel ALN Kit and the ALN test in the other product.

### 16. LIMITATIONS

1. Most strains of *Bacteroides* are ALN-positive, however, *B. ureolyticus* is ALN-negative. A spot indole and PYR test can be used to differentiate *B. ureolyticus* from *Fusobacterium* spp.; *B. ureolyticus* is negative with both tests.
2. This test is only part of the overall scheme for identification of anaerobic Gram-negative bacilli.

Further biochemical testing may be required for definitive identification. Consult appropriate references as necessary.<sup>1-4</sup>

3. Subculture isolates older than 72 hours (3 days) and perform the ALN test the next day.<sup>5</sup>

### 17. BIBLIOGRAPHY

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3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
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### 18. PACKAGING

REF R211356, ALN Disk..... 25 Disks/Vial

### 19. SYMBOL LEGEND

	Catalogue Number
	<i>In Vitro Diagnostic Medical Device</i>
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
	For Laboratory Use Only
	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

ATCC is a registered trademark of American Type Culture Collection.

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