remel
PYR/ESCULIN DISK

REF R21138 ..... 25 Disks/Vial

1. INTENDED USE

Remel PYR/Esculin Disk is a reagent-impregnated disk recommended for use in qualitative procedures for rapid, presumptive identification of group A *streptococci* and *enterococci*.

2. SUMMARY AND EXPLANATION

In 1981, Godsey et al. described a test to differentiate group A *streptococci* and *enterococci* from other *streptococci* based on their ability to cleave L-pyrrolidonyl-β-naphthylamide (PYR).[1] In 1982, Facklam et al. used the PYR test in conjunction with the CAMP and esculin hydrolysis tests to presumptively identify *streptococci*.[2,3] Ellner et al. described a colorimetric method for the PYR test using filter paper strips containing PYR substrate.[4] Using a rapid spot test, Edberg et al. demonstrated the hydrolysis of esculin to esculetin by utilizing the loss of fluorescence as an indicator of esculin hydrolysis.[5]

3. PRINCIPLE

The enzyme pyrrolidonyl peptidase hydrolyzes the substrate L-pyrrolidonyl-β-naphthylamide (PYR) to produce β-naphthylamine. Following addition of N,N-dimethyl-aminocinnamaldehyde (PYR Reagent) a red color is formed. Esculin is hydrolyzed at the β-glucose linkage to yield two products, esculetin and glucose. Esculin fluoresces under ultraviolet light at 360 nm, whereas the hydrolysis product, esculetin, does not. Both reactive substrates, PYR and esculin, are lyophilized in disk form for stability.

4. REAGENTS

Reactive Ingredients: L-pyrrolidonyl-β-naphthylamide
Esculin

5. 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.

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. Allow product to equilibrate to room temperature before use. Do not incubate prior to use. Protect from light.

7. PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from white, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) 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, TRANSPORT

Specimens should be collected and handled following recommended guidelines.[6]

9. 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) Longwave ultraviolet light, (8) Applicator sticks, (9) PYR Reagent (REF R21258).

10. PROCEDURE

The test isolate should be removed from an 18-24 hour culture. Ensure that the culture is pure or that selected colonies are well isolated from surrounding organisms.

1. Implement appropriate procedures to verify that the test isolate is a *streptococci* or *enterococci*.
2. Place the PYR/Esculin Disk on the surface of the agar plate.
3. Using a sterile loop or an applicator stick, inoculate a small area of the disk with isolated colonies.
4. Replace the lid of the Petri dish and incubate aerobically for 15 minutes at 35-37°C. Do not invert the plate.
5. Using a long wave ultraviolet light, observe the disk for fluorescence in a darkened room.
6. After interpreting the esculin reaction, add one drop of PYR Reagent to the disk and observe for a pink to red color development within 1-2 minutes.

Note: If only performing the PYR test, organism-inoculated disks may be incubated at room temperature for 5 minutes prior to adding the PYR Reagent. If only performing the esculin test, as for anaerobes, organism-inoculated disks must be incubated for 15 minutes at 35-37°C.[7]

11. INTERPRETATION

PYR:	Positive Test -	Pink to red color within 1-2 minutes
	Negative Test -	No color within 1-2 minutes
Esculin:	Positive Test -	Loss of fluorescence
	Negative Test -	Bright blue fluorescence

12. QUALITY CONTROL

All lot numbers of PYR/Esculin Disk 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	
		PYR	ESC
Enterococcus faecalis ATCC® 29212	Aerobic, 15 min. @ 35-37°C	+	+
Streptococcus agalactiae ATCC® 12386	Aerobic, 15 min. @ 35-37°C	-	-
Streptococcus bovis ATCC® 9809	Aerobic, 15 min. @ 35-37°C	-	+
Streptococcus pyogenes ATCC® 19615	Aerobic, 15 min. @ 35-37°C	+	-

13. LIMITATIONS

1. Perform the fluorescence test for esculin hydrolysis before addition of PYR Reagent which will mask fluorescence of the disk.
2. Some viridans *streptococci* are positive for esculin hydrolysis. Further biochemical testing may be required to differentiate group D *streptococci* from esculin-positive viridans *streptococci*.[6]
3. This test is only part of the overall scheme for identification of *streptococci*. Additional biochemical and/or serological testing may be required for definitive identification. Consult appropriate references for further instructions.[6]

14. BIBLIOGRAPHY

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15. PACKAGING

REF R21138, PYR/Esculin Disk ..... 25 Disks/Vial

16. SYMBOL LEGEND

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
LAB	For Laboratory Use Only
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

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