

# Product Information Leishman

## Intended Use

Leishman's stain is used for staining of blood and bone marrow. It is generally used to differentiate and identify leucocytes, malaria parasites, and trypanosomas (unicellular parasitic protozoa). It is based on a mixture of methylene blue and eosin.

## Principle

The purple colour of cell nuclei, is due to molecular interaction between eosin Y and an azure B-DNA complex. The intensity of the staining depends on the azure B content and on the ratio azure B/eosin Y. The pH of the solutions, fixation and staining time can influence the staining result.

## Composition

Leishman contains Leishman stain and methanol.

## Stability and Storage

Leishman staining solution is stable for five years. The bottles must be kept closed. The advised storage temperature is 18 - 30°C. Used solutions and solutions that are past their shelf-life must be disposed of, according to local disposal guidelines.

## Procedure

- A. Method using a concentrated Leishman / Buffer solution
1. Prepare Sørensen buffer solution pH 7:  
Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L de-ionized water.  
Do not use tap water.
  2. Prepare a cytospin slide or smear by gently lowering the slide into a coplin into a coplin jar of acetic alcohol (3% acetic acid in 95% methanol), fix for 1 minute, wash off the fixative with distilled water and drain.
  3. Prepare the Leishman / Buffer solution  
Dilute 50 ml Leishman solution with 150 ml Sørensen buffer solution pH 7.  
Mix and allow to stand for 10 minutes.
  4. Proceed according to the below

Reagent sequence	Minutes
Undiluted Leishman	1
Leishman / Buffer solution	7
Rinse in distilled or de-ionized water	Few seconds
Buffer	2
Rinse in distilled or de-ionized water	Few seconds
Dry	

- B. Method using a more diluted Leishman / Buffer solution

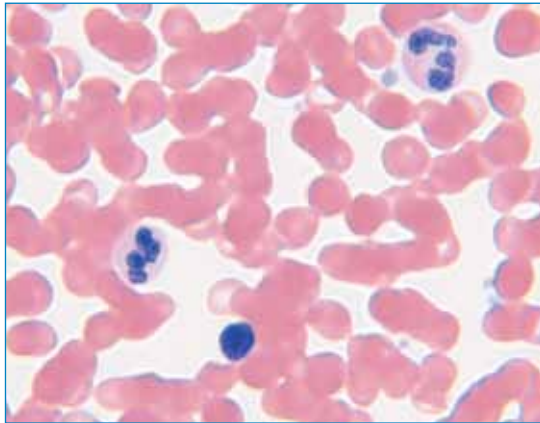
1. Prepare Sørensen buffer solution pH 7  
Dilute 100 ml of the concentrated Sørensen phosphate buffer with 1.9L de-ionized water.  
Do not use tap water.
2. Prepare a cytospin slide or smear by gently lowering the slide into a coplin jar of into a coplin jar of acetic alcohol (3% acetic acid in 95% methanol), fix for 1 minute, wash off the fixative with distilled water and drain.
3. Prepare the Leishman / Buffer solution  
Dilute 50 ml Leishman solution with 250 ml Sørensen buffer solution pH 7.  
Mix and allow to stand for 10 minutes.
4. Proceed according to the below

Reagent sequence	Minutes
Undiluted Leishman	3
Leishman / Buffer solution	6
Buffer	1
Buffer	1
Rinse in running tap water	2
Dry	

**Note:** The times as listed in the tables are approximate and can be adjusted to suit personal preferences. Staining solutions will lose their staining power when heavily used and the staining times should be longer or fresh solutions should be used.

## Performance Characteristics

Red blood cells	: red to yellowish red
Neutrophils	: dark purple nuclei, pale pink cytoplasm, reddish-lilac small granules
Eosinophils	: blue nuclei, pale pink cytoplasm, red to orange-red large granules
Basophils	: purple to dark blue nucleus, dark purple, almost black large granules
Lymphocytes	: dark purple to deep bluish purple nuclei, sky blue cytoplasm
Platelets	: violet to purple granules
Parasites (Leishmania, malaria, etc.)	: Dark blue – black



• Leishman stain (100x)

### Troubleshooting

Red cells are blue and nuclei are deeply stained

- Prolonged staining
- Inadequate washing
- Excessively alkaline stain or stain/buffer mixture
- pH of buffer too high

Excessive redness of red cells and poor staining of nuclei

- Inadequate staining
- Excessive washing
- Excessively acid stain or stain/buffer mixture
- pH of buffer too low

### Colour intensity

Varying times may influence the colour intensity:

Step 1 Leishman	Step 2 Leishman / Buffer solution	Step 3 Sørensen buffer	Colour intensity nucleus	Colour intensity Cytoplasm
See procedure.				
> procedure times			++	++
< procedure times			--	--
	> procedure times		--	--
	< procedure times		++	++
		> procedure times	--	--
		< procedure times	++	++

### Ordering Information

Product	Product Number	Packaging
Leishman	3879.1000	1 liter
Sørensen buffer concentrate	3716	10 x 100 ml



Phillipsburg, NJ 9001:2008 & 14001:2004  
 Paris, KY 9001:2008  
 Mexico City, Mexico 9001:2008  
 Deventer, the Netherlands 9001:2008, 14001:2004 & 13485:2003

Gliwice, Poland 9001:2008 & 17025:2005  
 Selangor, Malaysia 9001:2008  
 Dehradun, India 9001:2008, 14001:2004 & 13485:2003  
 Mumbai, India 9001:2008 & 17025:2005

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### Ordering Information and Assistance

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