# Microscope reagent kit

## Contents

* 1% Congo Red
* 1% Phloxine B
* Safranin
* Lactophenol Cotton Blue
* Melzer's Reagent
* Potassium hydroxide 2%

## Introduction

Stains are useful for enhancing the contrast in a microscopic image and highlighting particular tissue structures. Being able to view these structures and seeing the staining reactions can help differentiate species epically for families that have very similar looking species like *Russula* and *Lactarius*. Complete instructions for using these stains are beyond the scope of this document but there are a great many resources on the web that will help you get started.

## Usage

### Potassium hydroxide 2%

Potassium hydroxide 2% aqueous solution is used as a mounting medium it is often combined with a stain such as Phloxine B.

### 1% Congo Red

A general-purpose stain for looking at the fine details of hyphal structures, it will stain the Hyphae walls and contents.

### 1% Phloxine B

Michael Kuo’s go to stain. Stains only the Hyphae contents.

Congo red and phloxine can be mixed together before staining, and for best results use one drop of combined stain and one drop of ammonium hydroxide, and wash away surplus stain with ammonium hydroxide.

### Safranin

A stain that produces red cell nuclei.

### Lactophenol Cotton Blue

Also known as aniline blue. This stains chitin, making such structures as spore ornamentation show up much more clearly than they do with most other stains. Can also stain just the Hyphae contents but stains debris and bacteria as well. Lactophenol Cotton Blue also works well to stain the structure in ascomycetes.

### Melzer's Reagent

One of the most important reagents in a mycologist’s toolbox. Contains chloral hydrate and iodine. The chloral hydrate acts as a clearing agent, bleaching and improving the transparency of various dark-colored microscopic materials. The iodine reacts with starchy substances to produce an intense blue-black color, and fungal spores that contain starch are referred to as ‘amyloid’. The following reactions with spores or fungal material can be observed:

* Amyloid or Melzer's-positive reaction, in which the material reacts blue to black.
* Pseudoamyloid or dextrinoid reaction, in which the material reacts brown to reddish-brown.
* Inamyloid or Melzer's-negative, in which the tissues do not change color, or react faintly yellow-brown.

Among the amyloid reaction, two types can be distinguished:

* Euamyloid reaction, in which the material turns blue without potassium hydroxide (KOH)-pretreatment.
* Hemiamyloid reaction, in which the material turns red in Lugol's solution, but shows no reaction in Melzer's reagent; when KOH-pretreated it turns blue in both reagents.

## Other resources

There are a number of websites that detail staining reactions and uses for the reagents.

<http://www.mushroomexpert.com/microscope.html>

<http://www.britmycolsoc.org.uk/mycology/microscopy/reagents/>

<http://fungus.org.uk/nwfg/chemdec99.htm>

<http://www.first-nature.com/fungi/~microscopy.php>