

# AG-CALCOFLUOR WHITE STAIN KIT(IFU)

#### PRINCIPLE:

AstraGene's Calcofluor White Stain is **a** non-specific fluorochrome stain that binds with cellulose and chitin. Upon excitation with long wave ultraviolet light, this compound delineates the cell walls of cellulose-containing organisms. Prior to staining with calcofluor white a clearing agent, such as potassium hydroxide, is added to the specimen to dissolve tissue cells.

#### **APPLICATIONS:**

AstraGene's Calcofluor White Stain Kit is recommended for use in qualitative procedures as a rapid, non-specific fluorochrome stain for the initial microscopic detection of fungal elements, yeasts, Acanthamoeba cysts, microsporidia, and Pneumocystis carinii in clinical specimens.

### **PACKAGE CONTENTS:**

Description	Catalogue number	Quantity
Reagent -A (10% KOH solution)	AG/Stain/CW/22/01	200ml
Reagent -B (0.1 % Calcofluor White Solution)	AG/Staff/CW/22/01 200ml	200ml

### **STORAGE & STABILITY:**

Prepacked reagents are stored at room temperature and are stable until the mentioned expiry date.

## SPECIMEN COLLECTION AND HANDLING:

- For clinical samples, food, dairy samples, and water samples follow appropriate techniques for handling specimens as per established guidelines.
- After use, contaminated materials must be sterilized by autoclaving before discarding.

#### **MATERIAL REQUIRED:**

- Inoculating loop or needle, swabs, collection containers.
- Glass slides, coverslips,
- Clinical Samples
- Fluorescent microscope, (Blue Filter)
- Quality control organisms

## STAINING PROCEDURE:

## 1. Fungal elements and yeasts:

- Place the specimen on a clean glass slide.
- Add 1 drop of Reagent A and gently mix.
- Add 1 drop of Reagent B and mix.
- Cover with a clean glass coverslip and examine the slide using a fluorescent microscope. Observe fluorescence and typical morphology.

## 2. Microsporidia:

- Although the most common specimen is a fresh or preserved (formalin or SAF) stool specimen, other specimens such as tissues, duodenal aspirates, concentrated urine, sputum, CSF, nasal discharge, Bronchoalveolar Lavage (BAL), and conjunctiva are also appropriate. Place a thin specimen (10 µl) on a clean glass slide and heat-fix on a slide warmer at 60°C until dry.
- Fix slide in methanol for 2 minutes.
- Add 1 drop of Reagent A and gently mix.
- Add 1 drop of Reagent B and mix.
- Stain for one minute and remove excess stain by rinsing in demineralized water. Cover slide with a clean glass coverslip and examine the specimen using a fluorescent microscope. Observe fluorescence and typical morphology.

#### 3. Pneumocystis carinii:

- The specimens of choice are concentrated BAL (10-25 µl) or tissue samples. Decreased sensitivity is observed with induced sputum. Place specimen on a clean glass slide and air dry.
- Fix slide in methanol for 2 minutes.
- Add 1 drop of Reagent A and gently mix.
- Add 1 drop of Reagent B and mix.
- Stain for one 1minute. Remove excess stain by rinsing in demineralized water. Cover slide with a clean glass coverslip and examine the specimen using a fluorescent microscope. Observe for typical morphology.



## **RESULT INTERPRETATION:**

Fungal elements, yeasts	Bright apple-green fluorescence with typical morphology
Microsporidia	Intestinal microsporidia spores range in size from 0.9-1.5 μm to 1.2-2.0 μm, cell
	wall is brightened but staining is not specific
Pneumocystis carinii	Brilliant apple green fluorescence, cysts are 5-8 µm in diameter and contain up to
	8 crescent or pleomorphic shaped sporozoites; cell wall and double parenthesis
	structures inside the cysts stain intensely
Bacteria	Weak to no fluorescence; typical coccoid or bacillary shape

## **PRECAUTIONS:**

- 1. The kit should only be used for *in vitro* detection.
- 2. All operations must be carried out in strict accordance with the instructions.
- 3. All the samples need to be considered as biologically hazardous and handled accordingly.

# **SYMBOLS:**



Date of manufacture



Use-by-date



Do not use if package is damaged



Manufacturer



Batch Code



Refer to the instructions



ISO



GMP



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