

# PyroColor Diazo Reagents

Cat #DIA150-MP

## Instructions For Use

For use with Endospeccy® ES-50M Kit, Toxicolor® LS-50M Kit.



ASSOCIATES OF  
**CAPE COD**  
INCORPORATED  
124 Bernard E. Saint Jean Drive • E. Falmouth, MA 02536 USA  
telephone: (508) 540-3444 • www.acciusa.com



PN002248 Rev1

October 2010

### Materials included with kits:

No.	Description	Volume
1A	Hydrochloric (HCl) solution [ <i>for dissolving</i> (1)]	4.0 mL x 2 vials
1	Sodium nitrite	1.65 mg x 2 vials
2	Ammonium sulfamate	12 mg x 2 vials
3	<i>N</i> -(1-Naphthyl) ethylenediamine dihydrochloride	2.8 mg x 2 vials
3A	<i>N</i> -Methyl-2-pyrrolidone solution [ <i>for dissolving</i> (3)]	4.0 mL x 2 vials

### Preparation of the diazo-coupling reagents:

Using the reagents supplied with the kit:

1. Add the total volume (4.0 mL) of HCl solution (1A) to the vial of sodium nitrite (1).
2. Reconstitute the vial of ammonium sulfamate (2) with 4.0 mL of distilled water.
3. Add the total volume (4.0 mL) of *N*-Methyl-2-pyrrolidone solution (3A) to the vial of *N*-(1-Naphthyl) ethylenediamine dihydrochloride (3).
4. Mix and dissolve the contents by gently swirling the vials to prepare solutions (1), (2), and (3).

### Procedure:

Please read the assay package insert prior to performing a test.

1. After assay incubation is completed, immediately add 50  $\mu$ L of Solution (1) to each well and gently shake the plate to mix. Promptly add solution (1) to all wells to ensure that all samples incubate for the same period of time.
2. Add 50  $\mu$ L of Solution (2) to each well and shake plate to mix.
3. Add 50  $\mu$ L of Solution (3) to each well and shake plate to mix. Full color (magenta) should develop immediately.
4. Place the plate in a dual-wavelength microplate reader and measure absorbance at 545 nm using a reference wavelength of 630 nm.

### Precautions in use and handling:

Please read this package insert carefully before using the set.

1. All reagents contained in this set are intended for research purposes only. It is not intended for diagnosis of humans or animals.
2. Care should be exercised in handling all reagents in the PyroColor DIA150-MP kit. Material Safety Data Sheets (MSDS) available upon request.