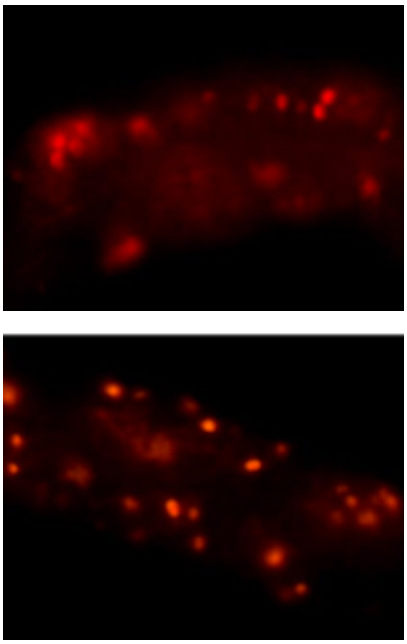
NemaMetrix

RediStain™ WormDye Lyso Reagent

SKU: DYE9440 SIZE: 500 UL (100 USES)

<-20°C		4mg/mL
STORAGE UPON RECEIPT	**PROTECT FROM LIGHT**	**PACKAGED CONCENTRATION**
650 / 526nm RNA / DNA	460 / 500nm RNA / DNA	Water
EMISSION	**EXCITATION**	**SOLUTE**



Endocytic vesicles marked by feeding live animals Acridine Orange[3].

Description

RediStain™ WormDye Lyso reagent is a vital stain that can be used to stain apoptotic corpses in the *C. elegans* gonad in live worms. It is also used as a lysosomal marker. RediStain™ WormDye Lyso reagent is chemically identical to Acridine Orange.

Standard Worm Staining Protocol

1. PREPARE WORMS

1.1 Pipette 1 mL of M9 onto plate containing *C. elegans*. Swirl plate gently and transfer M9 and worms into EpiTube

1.2 Wash worms 3x

1.2.1 Allow worms to settle or centrifuge at 6000 RPM for 2 minutes

1.2.2 Remove supernatant, leaving worms in pellet at bottom of the EpiTube

1.2.3 Add 1 mL M9

1.2.4 Repeat Steps 1.2.1-1.2.3 x2

1.2.5 After the final rinse, remove as much liquid as possible, leaving only worm pellet

2. DILUTE

2.1 Dilute RediStain™ Lyso reagent immediately prior to use

2.1.1 Thaw at room temperature

2.1.2 Add 5 µL of RediStain™ Lyso reagent to 1 mL of M9

2.1.3 Pipette to mix

3. INCUBATE

3.1 Add diluted RediStain™ Lyso reagent to the freshly washed worms (step 1.3)

3.2 Incubate for 20-30 minutes at room temperature and away from light

4. WASH

4.1 Remove RediStain™ Lyso reagent, leaving worms in a pellet at the bottom of the EpiTube

4.2 Rinse in M9 buffer at least 3 times (see Step 1.2)*. Residual stain may obscure fluorescent signal.

**Alternatively, transfer worms to a fresh plate, and let crawl on a bacterial lawn for approximately 1 hour to destain. (If destaining worms on plate repeat steps 1-1.24, ending with rinsed worms in EpiTube.)*

5. USE

5.1 Image worms immediately.

Protocol for Simultaneous RediStain imaging and EPG in ScreenChip System

1. PREPARE WORMS

1.1 Pipette 1 mL of M9 onto plate containing *C. elegans*. Swirl plate gently and transfer M9 and worms into EpiTube

1.2 Wash worms 3x

1.2.1 Allow worms to settle or centrifuge at 6000 RPM for 2 minutes

1.2.2 Remove supernatant, leaving worms in pellet at bottom of the EpiTube

1.2.3 Add 1 mL M9

1.2.4 Repeat Steps 1.2.1-1.2.3 x 2

1.2.5 After the final rinse, remove as much liquid as possible, leaving only worm pellet

2. PREPARE M9-5HT SOLUTION IMMEDIATELY PRIOR TO USE

2.1 Prepare 1000ul 10mM Serotonin in M9

2.2 Vortex or invert until solution is fully mixed

3. DILUTE

3.1 Add 5 µL of RediStain™ Lyso reagent to 1000uL of 10mM 5HT

3.2 Pipette to mix

4. INCUBATE

4.1 Add diluted RediStain™ Lyso reagent-10mM 5HT solution to the freshly washed worms and incubate for 20-30 minutes at room temperature in the dark

5. WASH

5.1 Remove RediStain™ Lyso reagent-10mM 5HT, leaving worms in a pellet at the bottom of the EpiTube

5.2 Rinse in freshly prepared 10mM 5HT in M9 buffer at least 3 times (see Step 1.2). Residual stain may obscure fluorescent signal.

6. USE

6.1 Load worms into ScreenChip, record EPG data, and image worms immediately

References

1. Hersh, B. M., Hartwig, E., and Horvitz, H. R. (2002). **The *Caenorhabditis elegans* mucolipin-like gene cup-5 is essential for viability and regulates lysosomes in multiple cell types.** *Proc. Natl. Acad. Sci.* USA 99, 4355–4360.
2. Clokey, G. V., and Jacobson, L. A. (1986). **The autofluorescent "lipofuscin granules" in the intestinal cells of *Caenorhabditis elegans* are secondary lysosomes.** *Mech. Ageing Dev*. 35, 79–94.
3. Kang J, Shin D, Yu JR, Lee J. (2009) **Lats kinase is involved in the intestinal apical membrane integrity in the nematode *Caenorhabditis elegans*.** *Development*. Aug;136(16):2705-15. doi: 10.1242/dev.035485.

About NemaMetrix

NemaMetrix Inc. specializes in developing and manufacturing devices, consumables, and software for automatic worm screening and phenotyping.

The company's mission is to enable scientists and researchers around the world to better understand human diseases and explore potential treatments for high-impact disorders such as Alzheimer's disease, and ALS (Lou Gehrig's Disease), and cardiac arrhythmias by offering a more affordable and rapid system that supplements the traditional mouse model. Please visit our website for the most up to date information.

Learn more at www.nemametrix.com/about-us

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