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PRODUCT DATA SHEET

Pierce D-Luciferin, Monosodium Salt, 1g

Product Number: 88292 Lot Number: YL383116 TEST: **SPECIFICATION: RESULT:** Color: Slightly yellow to yellow Pass Molar Extinction Coefficient: \geq 17500 L/(mol cm) at 381-387nm 17750.0 at 384nm Molar Extinction Coefficient: \geq 6000 L/ (mol cm) at 282-286nm 6159.0 at 284nm $\leq 8.0\%$ Water Content (K. Fischer): 63% Purity (HPLC): \geq 99.0 area% 99.4 area-% FTIR: Must correspond to reference spectrum Pass

Storage: Upon receipt store product at -20°C. Product is shipped at ambient temperature.

Thermo Scientific D-luciferin is the substrate for firefly luciferase which catalyzes the oxidation of luciferin to oxyluciferin in the presence of ATP and magnesium, resulting in bioluminescence. Light output captured using a luminometer can be correlated with the amount of Firefly luciferase protein produced and used to determine the activity of the promoter driving Firefly luciferase expression.

Important Product Information

- D-Luciferin salts can be used with any existing reporter assay or ATP assay system.
- D-Luciferin salts are soluble in water or aqueous buffer up to 100mM. Stock solutions can be made in ATPfree ultrapure water and stored at -20°C.
- If testing for ATP, minimize all possible sources of ATP contamination by wearing gloves and using ATP-free containers. Use only sterile ATP-free water and reagents. Use autoclaved water for all reagent preparations.
- Follow luminometer manufacturer's instructions for the appropriate settings.
- Store any substrate or samples containing ATP only in polypropylene or glass to prevent loss due to binding of the tube material

Procedure for Determining Luciferin Concentration

- 1. Pipette 0.5mL of 0.5M carbonate buffer, pH 11.5 into a 1mL cuvette. Place cuvette in the spectrophotometer and zero the instrument.
- Add 15µL of D-Luciferin solution to cuvette in the sample side and mix well. Measure the absorbance at 381-387nm, using the wavelength listed in the Result column above. Adjust the amount of D-Luciferin solution added to the cuvette so the final absorbance ranges from 0.5 to 1.0.
- Calculate the sample's concentration using the extinction coefficient at 381-387nm listed in the Result column above. For example, if the solution has an extinction coefficient of 17,500M⁻¹cm⁻¹ and absorbance of 0.650 at 385nm, perform the calculations as follows:

 $0.650 = (17,500 \text{M}^{-1} \text{cm}^{-1})(1 \text{cm})$ (Concentration in cuvette)

Concentration in cuvette = $37.1 \mu M$

Concentration in sample = 0.

 $\frac{(37.1\mu M)(0.515mL)}{0.015 mL} = 1.27mM$

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