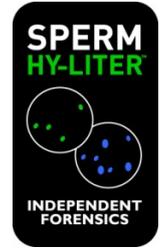


# SPERM HY-LITER™



## 1 M DTT Self Assembly KIT Protocol

### Introduction

Dithiothreitol (DTT), also known as Cleland's Reagent, is a water-soluble disulfide reducing agent used primarily in biochemistry and biology applications. It can be used to protect free SH-groups from oxidation during biochemical procedures and is frequently used to reduce the disulfide bonds in proteins. CAS Number: 3483-12-3; Molecular Weight: 154.253

### Intended Use

DTT is a mandatory component of **SPERM HY-LITER™** staining and is added to the Sample Preparation Buffer immediately before use. The Sample Preparation Buffer step is required for proper staining of sperm and is designed to expose sperm heads in all cell layers in the preparation.

**SPERM HY-LITER™** staining is critically dependent on the correct concentration and pH of the DTT solution. Independent Forensics' supplied DTT kit provides the necessary reagents to make 13 ml of 1M DTT, pH 8.0-8.5 as well as the microtubes and cryovial box for long-term storage of the prepared DTT solution. The protocol has been QA/QC tested for consistent, reproducible and strong staining of sperm from sexual assault evidence. It can be used for all biochemical procedures requiring disulfide reduction, including differential extraction, protein labeling, etc. DTT may be used for **SPERM HY-LITER™** staining at 1X or 10X final concentration (*i.e.*, 1  $\mu$ L or 10  $\mu$ L of 1 M DTT per two drops of Sample Preparation Buffer) – somewhat stronger staining can be observed using the 10X volume of DTT on most preparations.

### Reagents and Materials Provided

Plastic bag containing three tubes and plastic disposable transfer pipette

- Tube 1: 15 ml conical containing 2.005 g DTT
- Tube 2: 15 ml conical containing 10 ml DI H<sub>2</sub>O
- Tube 3: 1.5 ml eppendorf tube containing 1.1 ml KOH

One bag containing 100 0.5 mL micro tubes

Second bag containing lids for 100 0.5ml microtubes

Cryovial box for freezer storage

Technical Information Sheet

### Protocol for Assembly of 1M DTT

1. Add H<sub>2</sub>O from tube 2 to DTT in tube 1
2. Invert tube 1 until DTT is completely dissolved
3. Add KOH from tube 3 to tube 1 using disposable plastic transfer pipette, pH will be in the range of 8.0-8.5
4. Final volume will be approximately 13 ml.
5. Aliquot 100  $\mu$ l of the DTT solution into the provided microtubes and top with the provided lids
6. Insert the microtubes into the provided cryovial box and store at -20°C

Shelf Life: 2 years from date of manufacture

### Application

Each 0.5 mL micro tube is designed for single use only. Do NOT thaw and refreeze for future use. It has been observed that increased amounts of DTT can improve **SPERM HY-LITER™** fluorescent signals in samples demonstrating weak staining. Analysts, at their discretion, may increase the amount of 1 M DTT added to the Sample Preparation Buffer up to 10X, *i.e.*, 10  $\mu$ L DTT per 2 drops of Sample Preparation Buffer.