

Reducing trypan blue hazardous waste

Trypan blue has historically been a trusted stain for cell counts and viability measurements in cell and tissue culture facilities. Recently, however, trypan blue has been labeled a potentially hazardous chemical. In light of this labeling, the following simple protocol modifications can minimize the production of potentially dangerous waste:

- Reduce trypan blue concentrations
- Reduce required sample volumes
- Eliminate high-volume diluting wash steps

The Countess™ Automated Cell Counter, which is used with trypan blue, functions effectively with lower than conventional concentrations of this chemical. The following modifications outline how to prepare and use a 0.1% concentration of trypan blue with the Countess™ Automated Cell Counter, as well as how to reduce sample volumes and prepare for safer disposal.

Reduce staining concentration of trypan blue to 0.1%

The Countess™ Automated Cell Counter is automatically calibrated for 0.4% trypan blue. By making a stock concentration of 0.1% trypan blue and recalibrating the instrument according to the protocol below, users can safely work with lower concentrations of this dye.

Prepare 0.1% trypan blue staining solution

Dilute trypan blue

Mix one part 0.4% trypan blue with three parts 1X phosphate-buffered saline (PBS), pH 7.4 (Cat. no. 20012-027), to make a 0.1% solution.

For example, mix 250 μL of 0.4% trypan blue with 750 μL of 1X PBS.

Calibration

1. Mix one volume of 0.1% trypan blue with an equal volume of buffer or water.
2. Load 10 μL of this solution into a Countess™ Chamber Slide.

3. Insert the slide, sample side first, into the Countess™ slide port.
4. In the “settings” menu, press “calibrate”. The instrument will take about 30 seconds to calibrate.
5. When prompted, press “restart”.
6. The Countess™ Automated Cell Counter is now calibrated for 0.1% trypan blue and ready to count cells.

Reduce the working volume

Reducing the pipetting volume can eliminate excess trypan blue. For example, standard protocols suggest mixing 10 μL trypan blue with 10 μL cell suspension and loading 10 μL , leaving a 10 μL excess that needs disposal.

However, the following protocol produces no excess working solution:

Counting

1. Mix 5 μL cell suspension with 5 μL of 0.1% trypan blue and load the entire 10 μL into a Countess™ Chamber Slide.
2. Insert the slide into the instrument, loaded side first. The instrument reads only one chamber at a time.
3. Use the zoom function and adjustment knob to focus the image and bring the objects into proper position.
4. Press “count cells” to begin acquiring and analyzing the image.

Reduce high-volume wash steps

Traditional glass hemocytometers require cleaning after each count, and are usually washed with alcohol. The entire wash solution should be captured and disposed of as hazardous, flammable material.

With the disposable Countess™ Cell Counting Chambers, the solution is contained under a fixed coverslip. By disposing of the entire chamber, any potentially hazardous wash steps are eliminated. The entire sample can then be disposed of according to local regulations.