LSC in Practice

Solubilization of Mouse Liver for Microplate Counting

Problem

One of PerkinElmer's field sales engineers required help to develop a method for the solubilization of mouse liver that must be compatible with PerkinElmer's MicroScint™-40 cocktail (PerkinElmer part number 6013641) for sample counting on a PerkinElmer TopCount® microplate scintillation and luminescence counter. The researcher's laboratory would use PerkinElmer's 24-well PicoPlate™ (PerkinElmer part number 6005163). The major concerns included compatibility between the solubilizer and the specialty MicroScint cocktail, and identification of a bleaching agent for the colored liver samples. The isotope of interest was ^{90}Y.

Discussion

Essentially, the researcher's goals can be achieved. However, there are certain precautions and restrictions that must be observed when performing the sample preparation.

Utilizing the following chemicals, we performed several tests and refined our standard solubilization procedures to accommodate the use of MicroScint-40 and the PicoPlate:

- SOLVABLE™ (PerkinElmer part number 6NE9100) for solubilizing the mouse liver
- Hydrogen peroxide for decolorizing
- MicroScint-40 for counting

SOLVABLE was selected as the digesting agent because it solubilizes liver rapidly and produces less color after solubilization than other commercially available products. Up to 100 mg of mouse liver may be dissolved within 1 1/2 hours at 50 °C to 60 °C by 1.0 mL of SOLVABLE.

As a point of caution, the digestion should not be performed in the selected PicoPlate, which is made out of BAREX™. Although BAREX is a good choice for resistance to a wide range of chemical agents, it does have a low tolerance for elevated temperatures and will distort above 45 °C. In addition, the secondary hydrogen peroxide decolorization steps will probably result in frothing and cause an overflow out of the well.

The following procedure was optimized for this application:

1. Add up to 100 mg liver to a 20 mL glass vial.
2. Add 1.0 mL SOLVABLE and heat at 50 °C to 60 °C for 1 to 2 hours or until all the liver is solubilized.
3. Add 200 µL hydrogen peroxide in two 100 µL portions with gentle swirling between additions.
4. After the reaction has subsided, heat again to 50 °C to 60 °C for about 15 minutes to complete the reaction.
5. Cool to room temperature.
6. Take an aliquot (100 µL to 200 µL) and add this to a well together with 1.50 mL MicroScint-40; seal.
7. Mix using an orbital shaker before counting.
8. Allow to temperature and light adapt for 15 to 30 minutes before counting to allow any luminescence to decay. Alternatively, ensure that the window setting excludes the low energy region and therefore excludes the luminescence; this should be possible since ^{90}Y is a high energy emitter.

Recommendation

We strongly recommend carrying out the solubilization steps, listed above, in a vial and then taking aliquots for counting as this will ensure complete solubilization and prevent damage to the microplate. The use of aliquots will allow counting in duplicate or triplicate and still reserve sufficient sample to

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices

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