BIOMEDICAL RESEARCH SERVICE CENTER

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Lactate Dehydrogenase (LDH) Staining Kit (Cat #: E-106)

COMPONENTS: LDH Staining Solution- 40 ml, store at -80ºC **(shield solution from light during assay)**
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**
5x Loading Solution- 0.5 ml, store at 4ºC

PRODUCT DESCRIPTION: Lactate dehydrogenase (LDH) consists of 4 subunits which may be of 2 different types: M (muscle) and H (heart), also known as A and B respectively. Five different isoenzymes are therefore possible, depending on the subunit composition. The LDH staining kit is based on the reduction of a tetrazolium salt to a formazan product, which exhibits an intense dark blue color. Since the intensity of the dark blue color is proportional to LDH activity, the staining kit can be used to delineate LDH isoform distribution of serum/plasma, cell/tissue extracts, and cell culture medium by non-denaturing agarose gel electrophoresis.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 - 2 mg/ml.

Agarose gel electrophoresis:

1. Prepare 1 liter Tris-Borate buffer by adding 10.8 g Tris and 5.5 g Boric acid to 1 liter dH_2O. Stir solution to dissolve contents. Store buffer at room temperature.
2. Prepare a 0.8% mini agarose gel (~5 cm x 6 cm) in Tris-Borate buffer, e.g. 0.16 g agarose in 20 ml buffer. Dissolve agarose by microwave heating (Caution HOT!). It is a good idea to microwave for ~30 sec, swirl solution and then continue towards a boil. Keep an eye on it to avoid over-boiling.
3. Pour agarose into a gel tray with the well comb in place, avoiding bubbles which will disrupt the gel. Let sit at room temperature for 20 − 30 minutes.
4. Remove the comb and place the agarose gel into the gel box (electrophoresis unit). Fill gel box with Tris-Borate buffer to cover gel. Do not over fill.
5. Mix 12 µl of each sample with 3 µl Loading Solution and load sample into the gel. Run gel at ~100V until the blue dye migrates near the bottom of the gel. Note: Black is negative and Red is positive. Run sample toward Red.

LDH staining:

1. Thaw LDH Staining Solution and keep solution on ice shielded from light. It is important to minimize the time that the solution is thawed. Freeze solution immediately after use.
2. Rinse gel in dH_2O for a few min and remove water completely. Place gel in the casting tray and add enough LDH Staining Solution to cover gel. Typically ~5 ml is sufficient to cover a 5 cm x 6 cm mini-gel. Seal the casting tray with plastic wrap and incubate in a humidified 37ºC incubator for at least 1 − 2 hours or until dark-blue LDH bands become visible. Five LDH bands from top to bottom may be visualized: LDH-5 (M_4), LDH-4 (M_3H_1), LDH-3 (M_2H_2), LDH-2 (M_1H_3) and LDH-1 (H_4). The gel is now ready for imaging.

Additional information:

- LDH Staining Solution contains DMSO and MTT. Avoid skin contact. Please refer to the product page of our website or contact us for MSDS information.