BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO
Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

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

COMPONENTS: LDH Staining Solution- 40 ml, store at -80ºC **(shield solution from light during use)**
5x Loading Solution- 0.5 ml, store at room temperature
10x Cell Lysis Solution- 25 ml, store at room temperature (**agitate vial to redissolve contents)**

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 can thus exist. The LDH staining kit is based on reduction of tetrazolium salt to a formazan product, which exhibits an intense dark blue color. 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. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~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 ~10 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 (~5 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. The suggested sample protein concentration range is 1-5 mg/ml. *Normalization of sample protein concentration is recommended.*

Agarose gel electrophoresis:

1. Prepare 1-liter Tris-Borate (TB) buffer by adding 10.8 g Tris and 5.5 g Boric acid to one liter of dH_2O. Stir solution to dissolve contents. Store buffer at room temperature.
2. Prepare a 0.8% agarose solution in TB, e.g., 0.16 g agarose in 20 ml TB. The volume can be adjusted based on the size of the gel casting apparatus. Dissolve agarose by microwave heating. *Caution HOT!*
3. Pour agarose solution into a gel tray with the well comb in place, avoiding air bubbles. Wait ~30 min.
4. Carefully remove the comb. Place agarose gel into an electrophoresis unit. Fill the gel box with TB to cover gel. *Do not over fill.*
5. Mix 12 µl of each sample with 3 µl of Loading Solution in a microtube. Run gel at ~100 volts until the bottom blue dye is near the end of the gel. *Black is negative and Red is positive. Run sample toward Red.*
6. Upon completion of electrophoresis, place gel in a small container and rinse gel in dH_2O for ~5 min. Remove water completely. The gel is now ready for staining.

LDH staining:

1. Thaw LDH Staining Solution and keep solution on ice and shielded from light. *Do not over thaw.* Freeze solution immediately after use.
2. Add enough LDH Staining Solution to cover gel. Typically, ~5 ml is sufficient to cover a 5 cm x 6 cm mini gel. Use more staining solution for a larger gel.
3. Seal the container with plastic wrap and incubate in a humidified 37ºC incubator with occasional agitation for 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).

Additional information:

- Heart tissue exhibits all five LDH bands and can be used as LDH reference.
- LDH Staining Solution contains DMSO and MTT. Avoid skin contact. Please refer to the product webpage or contact us for SDS information.