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

**COMPONENTS:**
- LDH Staining Solution: 40 ml, store at -80°C (store in aliquots after the first thawing)
- 10x Cell Lysis Solution: 25 ml, store at 4°C (swirl bottle briefly prior to pipetting)
- 5x Loading Solution: 0.5 ml, store at 4°C

**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 cell/tissue LDH isoform distribution by non-denaturing agarose gel electrophoresis.

**Plasma/serum/conditioned medium:** Sample can be mixed with Loading Solution and analyzed by agarose gel, e.g., 12 µl sample + 3 µl Loading Solution.

**Preparation of cell/tissue extracts:**
1. Wash ~10^6 cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80°C until use.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH2O. Add 50 – 100 µl 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm) and harvest supernatant for assay. For tissue extraction, weigh ~25 mg tissue and homogenize in 0.5 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4°C for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80°C.
3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 1 – 2 mg/ml.
4. Mix 12 µl lysate with 3 µl Loading Solution for agarose gel electrophoresis.

**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 dH2O. 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. Load sample premixed in Loading Solution into the well of 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 it on ice. It is important to minimize the time that the solution is thawed. Freeze solution immediately after use.
2. Rinse gel in dH2O 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 37°C incubator 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), LDH-4 (M,H), LDH-3 (M,H), LDH-2 (M,H) and LDH-1 (H). 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.