# Evans Blue Dye uptake

**-The Han Lab**

Evans Blue Dye (EBD) uptake assay is carried out to assess the muscle injury.

EBD is injected either into the tail vein of the mice or into the peritoneal cavity without anesthesia. For the assessment of EBD uptake into muscle fibers, intravenous administration of the dye was preferred. Nonspecific coloration of the diaphragm and the abdominal muscles was avoided by this route of injection.

Dissolve EBD 100 mg in 10 ml PBS (0.15 M NaCl, 10 mM phosphate buffer, pH 7.4) and sterilized by passage through membrane filters with a 0.2-um pore size. The concentration of the injected dye is 1% w/v.

Animals are injected with 50 Âµl of this solution per 10 g body wt.

3-6 h after injection, the mice are sacrificed and the animals are visually inspected for dye uptake into skeletal muscles, indicated by blue coloration.

Muscle sections from EBD-injected animals are incubated in ice-cold acetone at -20C for 10 min, washed 3 x 10 min with PBS, and mounted with Vectashield mounting medium (Vector Laboratories, Inc., Burlingame, CA).

By fluorescence microscopy analysis, EBD staining showed a bright red emission. Fiber counts of EBD-positive muscle fibers are done independently by two investigators on 7-um cryosections of dye injected mice.

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