

2.1. Mouse heart Evans blue dye (EBD) uptake assay

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A. OBJECTIVE

To quantitatively evaluate sarcolemmal integrity in the mouse heart.

B. CAUTIONS

- For all animal experiments, make sure to get approval from the Institute's Animal Care and Use Committee and follow NIH guidelines.
- EBD is considered a hazardous irritant and potential carcinogen.
- In the absence of β -isoproterenol injection, there is usually minimal EBD uptake in the mdx heart (Figure 1).

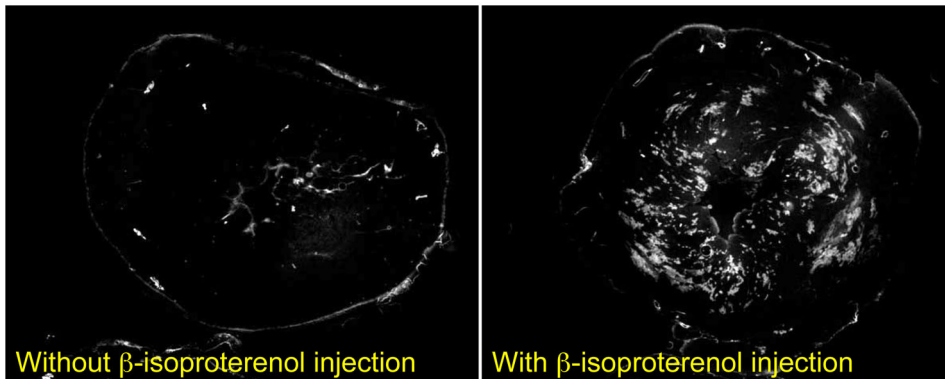


Figure 1. Mdx heart EBD uptake assay in the presence or absence of β -isoproterenol.

- We recommend use freshly prepared β -isoproterenol solution.
- Depending on the age of the mice, we use different protocols.
- For mdx mice that are older than 15 months of age, we notice a dramatic reduction of the EBD uptake. This observation is likely due to extensive fibrosis in the heart of aged mdx mice.

C. MATERIALS

- Evans blue dye. An azo dye with high affinity to albumin. It is named after Herbert McLean Evans (September 23, 1882 – March 6, 1971). It fluoresces with excitation peaks at 470 and 540 nm and an emission peak at 680 nm.
- PBS
- 0.2 μ m filter
- β -Isoproterenol.
- 1 ml insulin syringe for tail vein injection

RECIPES:

Evans blue dye (10 mg/ml):

1 gram Evans blue powder (Sigma, St Louis, MO, USA; catalog # E2129)

100 ml PBS
Filter through 0.2 μ m filter
Store in a cool, dry, well-ventilated area
Keep containers securely sealed

β -Isoproterenol solution (stock solution 10mg/ml; working solution 1 mg/ml):
100 mg (-) isoproterenol hydrochloride (Sigma, St Louis, MO, USA;
catalog # I 6504)
10 ml PBS
Filter through 0.2 μ m filter
Store in aliquots at -20°C
Dilute to 1 mg/ml at the time of use with sterile PBS

D. METHODS

Protocol 1: for mice that are younger than 2 months-of-age

1. Inject 300 μ l to 500 μ l EBD (200 μ g/g body weight) via the tail vein at 18 to 20 hrs before the first dose of β -isoproterenol injection.
2. 18 to 20 hrs after EBD injection, inject the 1st dose of β -isoproterenol at 500 ng/g body-weight.
3. 2 hrs after the 1st dose of β -isoproterenol, inject the 2nd dose of β -isoproterenol at 500 ng/g body-weight.
4. 2 hrs after the 2nd dose of β -isoproterenol, inject the 3rd dose of β -isoproterenol at 500 ng/g body-weight.
5. 2 hrs after the last dose of β -isoproterenol, euthanize the mouse.
6. Perfuse the heart thoroughly with PBS before remove the heart from the body.
7. Snap freeze the freshly dissected heart in 2-methybutane cooled liquid nitrogen in Tissue-Tek optimal cutting temperature compound (OCT) (Sakura Finetek Inc., Torrance, CA #4583).

Protocol 2: for adult mice ~ 6 to 8 months-of-age

1. Inject 300 μ l to 500 μ l EBD (200 μ g/g body weight) via the tail vein at 20~24 hrs before the first dose of β -isoproterenol injection.
2. 20~24 hrs after EBD injection, inject the 1st dose of β -isoproterenol at 500 ng/g body-weight.
3. 2 hrs after the 1st dose of β -isoproterenol, inject the 2nd dose of β -isoproterenol at 500 ng/g body-weight.
4. 2 hrs after the 2nd dose of β -isoproterenol, euthanize the mouse.
5. Perfuse the heart thoroughly with PBS before remove the heart from the body.
6. Snap freeze the freshly dissected heart in 2-methybutane cooled liquid nitrogen in Tissue-Tek optimal cutting temperature compound (OCT) (Sakura Finetek Inc., Torrance, CA #4583).

Protocol 3: for mice that are ~ 1-year-old

1. Inject 300 μ l to 500 μ l EBD (200 μ g/g body weight) via the tail vein.

2. 2 hrs after EBD injection, inject the 1st dose of β -isoproterenol at 800 ng/g body-weight.
3. 2 hrs after the 1st dose of β -isoproterenol, inject the 2nd dose of β -isoproterenol at 800 ng/g body-weight.
4. 2 hrs after the 2nd dose of β -isoproterenol, euthanize the mouse.
5. Perfuse the heart thoroughly with PBS before remove the heart from the body.
6. Snap freeze the freshly dissected heart in 2-methylbutane cooled liquid nitrogen in Tissue-Tek optimal cutting temperature compound (OCT) (Sakura Finetek Inc., Torrance, CA #4583).

E. EVALUATION AND INTERPRETATION OF RESULTS

1. Cut the heart into 8 to 10 μ m cross-sections.
2. Visualize EBD uptake under the Texas Red channel in a fluorescence microscope.

F. REFERENCES

Bostick B, Yue Y, Long C, Marschalk N, Fine DM, Chen J, Duan D (2009) Cardiac expression of a mini-dystrophin that normalizes skeletal muscle force only partially restores heart function in aged mdx mice. *Mol Ther* **17**: 253-261

Yue Y, Skimming JW, Liu M, Strawn T, Duan D (2004) Full-length dystrophin expression in half of the heart cells ameliorates beta-isoproterenol-induced cardiomyopathy in mdx mice. *Hum Mol Genet* **13**: 1669-1675