Alcian Blue – Alizarin Red Staining of Mouse Skeleton

Peichuan Zhang[1, 2*]

[1]Department of Biology, The Pennsylvania State University, University Park, PA, USA
[2]Present Address: Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA
*For correspondence: peichuan.zhang@ucsf.edu

[Abstract] Our lab has used the Alcian blue-Alizarin red staining method (Hanken and Wassersug, 1981) with certain modifications to characterize skeleton deformities in mice lacking Pek/Perk, encoding a translational control eIF2alpha kinase (Zhang *et al.*, 2002). Our protocol to conduct this experiment is described here.

Materials and Reagents

1. Neural buffered formalin (Sigma-Aldrich, catalog number: HT5014)
2. Alcian blue 8GX (Sigma-Aldrich, catalog number: A5268)
3. Trypsin (Sigma-Aldrich, catalog number: T1426)
4. Saturated sodium borate (Sigma-Aldrich, catalog number: S9640)
5. Alizarin red (Sigma-Aldrich, catalog number: A3882)
6. Thymol (EM Life Science, catalog number: TX0615-1)
7. Ethanol
8. Glacial acetic acid
9. Potassium hydroxide
10. Glycerol
11. KOH
12. Trypsin solution (see Recipes)

Equipment

1. Conical tube

Procedure

A. Adults:

1. Fix mouse skeleton in 10% neutral buffered formalin for at least 24 h.

2. Rinse the sample in ddH_2O O/N (1 h for embryos) with gentle shaking, and post-fix it in 70% ethanol.
   Note: At this point, samples can be stored in 70% ethanol for a long period.
3. Remove skins and internal organs carefully from the sample.
   Note: Remove all skins, even those on the small toes.
4. Stain the sample with 0.02% Alcian blue 8GX (prepared in ethanol/ glacial acetic acid, 7: 3) for 1 to 2 days.
   Note: Cartilage tissues will be stained blue.
5. Wash the sample with plain ethanol/ glacial acetic acid (7:3) for 1 h.
6. Soak the sample in 100% ethanol O/N, and then in ddH_2O for 1 to 2 days.
7. Treat the sample with 1.0% trypsin (prepared in water solution containing 30% saturated sodium borate) O/N.
8. Should limp and blue cartilage be readily observed at this point, proceed to stain the sample with Alizarin red (prepared in 0.5% KOH) O/N.
   Note: Add enough (no specific amount) saturated Alizarin red until the solution appears dark purple. Mineralized bones will be stained red.
9. Treat the sample with a gradient series of 0.5% KOH/ glycerol (*i.e.,* 2:1, 1:1, 1:2 and 100% glycerol, 2 days for each step), and store it in glycerol with a crystal of thymol.

B. Embryos:

1. A similar protocol can be used to stain embryos. To do this, fix embryos in 90% ethanol for at least 1 week.
2. Treat the sample with 0.01% Alcian blue 8GX for 3 days, and then perform rehydration through a gradient series of ethanol (70% ethanol, 2 to 3 h, twice; 40% ethanol, 2 to 3 h; 15% ethanol, 2 to 3 h; ddH_2O, until the sample sinks to the bottom of a conical tube). Treat the sample further with fresh 1% KOH for 1 to 2 days until it becomes clear.
3. Treat the sample with 0.001% Alizarin red for 2 to 3 days until the bone becomes purple.
4. Rinse the sample ~ 3 times in 1% KOH, several hours each time.
5. Treat the sample through a gradient series of glycerol-KOH (20% glycerol/ 1% KOH, 24 h; 50% glycerol/ 1% KOH, 24 h; 80% glycerol/ 1% KOH, 24 h; 100% glycerol, 24 h x 2).

Representative data

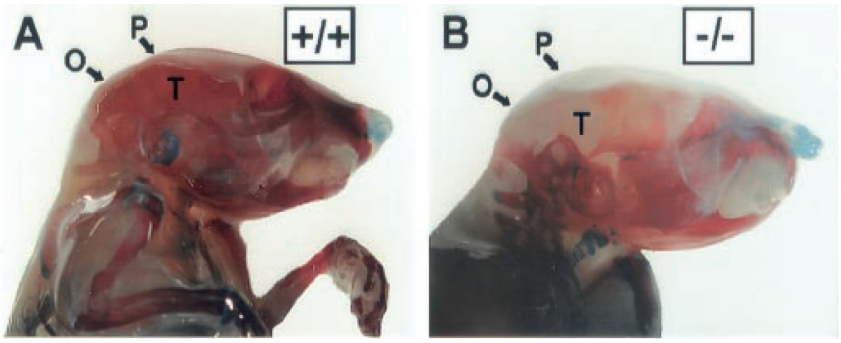

Figure 1. This figure is adapted from the original (Zhang *et al.*, 2002). Shown here is Alizarin Red (mineralized bone) and Alcian Blue (cartilage) skeletal staining of 18-day-old wild-type A and *Perk*$^{-/-}$ mutant mice B. The mineralization of the flat bones of the skull (P, parietal; O, occipital; T, temporal) is greatly reduced in the *Perk*$^{-/-}$ mutant mouse.

Recipes

1. Trypsin solution
   1.0% trypsin
   30% saturated sodium borate
   H_2O

Acknowledgments

This protocol was adapted from previously described work by Hanken and Wassersug (Hanken and Wassersug, 1981). PZ was supported by a research assistantship in the Cavener lab at the Pennsylvania State University.

References

1. Hanken, J. and Wassersug, R. (1981). The visible skeleton. *Funct Photogr* 16(4): 22-26.
2. Zhang, P., McGrath, B., Li, S., Frank, A., Zambito, F., Reinert, J., Gannon, M., Ma, K., McNaughton, K. and Cavener, D. R. (2002). The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. *Mol Cell Biol* 22(11): 3864-3874.

