

Barrier Function Assay

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[Abstract] This protocol serves to determine the integrity of the barrier function of (murine) epidermis. Defects in the barrier function lead to dehydration and infection in neonatal mice/humans. One possible way to assess epidermal barrier integrity is by a dye penetration assay as described hereunder. This assay should be done on unfixed, untreated tissues (e.g. formaldehyde- or glutaraldehyde-fixed) or on whole mouse embryos (E18.5). This protocol was adapted from Hardman (1998).

Materials and Reagents

1. E18.5 embryos from mice
2. Chilled methanol in water (25%, 50%, 75% and 100% MetOH)
3. 0.1% toluidine blue (in water) (Sigma-Aldrich)
4. Chilled 1x PBS (pH 7.4) (Sigma-Aldrich, catalog number: P4417) (see Recipes)

Equipment

1. Forceps
2. Camera

Procedure

1. Isolate uterus from pregnant females to obtain E18.5 embryos.
2. Euthanize embryos by immersion in ice cold PBS for 30 min.
3. At 18.5 days gestation, cull mother by cervical dislocation. Make a ventral incision and remove the uterine horn containing the embryos. To euthanize embryos, immerse entire uterus containing embryos in ice cold PBS for 30 min. Separate embryos and remove from uterus. Check that embryos have been successfully euthanized by pinching toe or

tail with forceps. If embryo responds, place back into ice cold PBS and check every 5 min. Once embryos are unresponsive/culled, proceed to the next step.

4. Finger/toe clip individual embryos for identification. This is especially important when crossing heterozygous mice. The digits can be used for genotyping the embryos.
5. Passage embryos through chilled methanol gradient, taking 2 min per step. It is crucial to have all solutions at 4 degrees centigrade. Keep solutions on ice throughout protocol.
 - a. 25% methanol in water
 - b. 50% methanol in water
 - c. 75% methanol in water
 - d. 100% methanol
 - e. 75% methanol in water
 - f. 50% methanol in water
 - g. 25% methanol in water
 - h. 100% water or PBS
6. Immerse embryos in 0.1% toluidine blue solution in water for 1-2 min on ice.
7. Destain embryos in PBS (pH 7.4) until a dye pattern emerges (in case of barrier defect) or until dye is washed away (in case of intact barrier) on ice.
8. Take pictures of individual embryos and if relevant genotype embryos. See also Figure 1 for some examples of intact barriers and barrier acquisition defects.

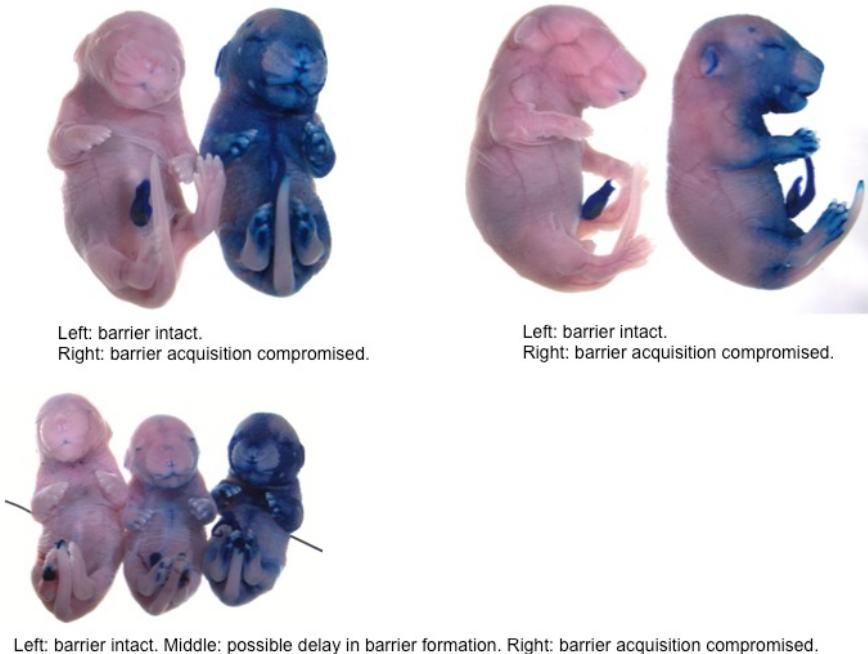


Figure 1. Examples of intact barriers and barrier acquisition defects

Recipes

1. 1x PBS (pH 7.4)
1 tablet per 200 ml dH₂O

Acknowledgments

This protocol was adapted from Hardman *et al.* (1998). Our work is supported by the Dutch Cancer Society (RUG 2012-5549), Stichting Kinder Oncologie Groningen (SKOG) and EMBO grants to FF.

References

1. Hardman, M. J., Sisi, P., Banbury, D. N. and Byrne, C. (1998). [Patterned acquisition of skin barrier function during development](#). *Development* 125(8): 1541-1552.