



### Protocol for Biostir AD ointment

#### The First Treatment

- 1) We recommend using female NC/Nga mice at 10 weeks old from Charles River Ltd. Co.
- 2) Shave their hair on the back and behind ears with a clipper and a shaver.
- 3) Remove hair completely with a depilatory.
- 4) Follow the explanations of the depilatory you use, and then wipe it off.
- 5) Apply 100 mg of Biostir AD to the shaved dorsal skin and both surfaces of each ear evenly.

#### From the second treatment on ...

- 1) If you can find the growth of hair on the back, you should shave them before every treatment.
- 2) After shaving hair, apply 150 $\mu$ l of 4 % sodium dodecyl sulfate solution on the shaved dorsal skin and both surfaces of each ear to make barrier disruption.
- 3) Dry the mice with a hair drier in cool mode.
- 4) Air –dry the solution for 2-3 hours.
- 5) Apply 100 mg of Biostir AD on the back and both surfaces of each ear evenly.

\* We recommend two treatments per week.

\*To make a chronic atopic dermatitis model, we recommend repeating six applications of Biostir AD.

#### Notes

If you apply Biostir AD on the back with growing hair, you might not achieve a high incidence rate.