

Preparation of cornified envelopes (CE)

(according to *Hohl D. et al., 1991, J. Biol. Chem. 266: 6626-6636* and *Jarnik M. et al., 1996, J. Cell Science 109: 1381-1391*)

- Preparation of epidermal sheets using NH₄-Thiocyanate solution (see P58).
- Alternatively, newborn total skin can be used.
- Cut tissue into pieces.
- Epidermal sheets / total skin are heated with stirring in CE-extraction buffer for 20-40 min at 95°C. Use 5 ml per epidermis and 10 ml per skin on a boiling water bath with vigorous stirring.
- Optional: Quick-freeze in LN₂ and store at -80°C until further processing.
- CEs are collected by centrifugation at 5000 x g for 15 min at RT.
- CEs are washed twice at RT with CE-extraction buffer containing 0.2% SDS.
- CEs are collected by centrifugation at 5000 x g for 15 min.
- Resuspend in 2% SDS solution.
- Adjust/determine CE concentration in a hemacytometer.
- Sonication (Branson 2210 cup sonicator) at 4°C, 20°C, 30°C, and 60°C.
- At various time points, remove CE aliquots from the sonicator, count with the hemacytometer and photograph.
- Final CE preps can be stored at 4°C for up to several weeks.
- Optional: Drop CE's (50µl) on a glass slide and embed in Mowiol.

CE-extraction buffer:

0.1 M	Tris/HCl, pH 8.5
2%	SDS
20 mM	DTT
5 mM	EDTA