

SDS-ELECTROPHORESIS WITH CLEANGELS USING THE BORATE-SDS BUFFER-KIT



2004 11/98

Best Results for Large Proteins and Glycoproteins

CleanGels are designed for horizontal electrophoresis with various buffer systems.

In addition to this multiple-purpose-quality the easy and stable storage-capability of these gels must be mentioned. CleanGels can be cut into customized pieces before rehydration.

The mostly used electrophoretic method for proteins is the SDS-electrophoresis. The main reasons for that: The migration speeds of the applied protein-SDS-myccells is relative to their molecular-weights; all proteins are running in the same direction (to the anode), and also the lipophylic proteins are soluble as SDS-myccells.

In vertical systems the Laemmli - buffer (1,2) is used, for the washed and dried CleanGels some optimizations had to be done. A discontinuous buffer system was developed using a TRIS-Cl buffer in the rehydration buffer so that the Laemmli-buffer samples could also be used.

In the electrode-buffer we introduced a TRIS-Borate buffer system giving also negative charges to the glycoproteins.

The resulting resolution is comparable

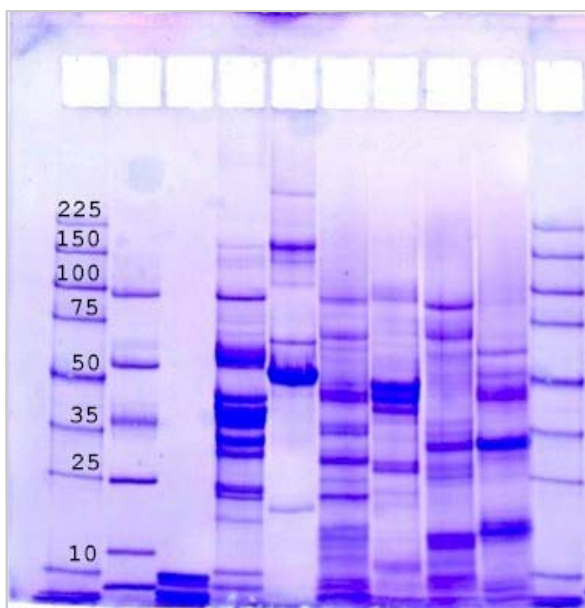
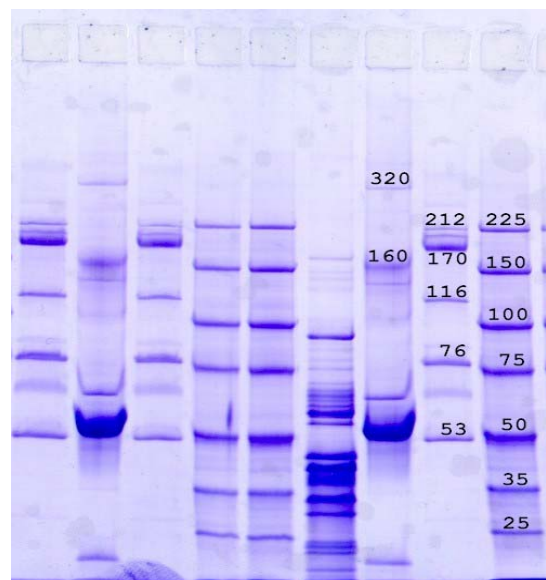


Fig.1: SDS-electrophoresis with a CleanGel 10% 21S using the Borate-SDS Buffer-Kit.



Right: CleanGel 7.5% 25S

Hardware

FlatTop horizontal chamber	ETC 1101-01
CleanPool	ETC 1003-20

Consumables

Borate-SDS Buffer-Kit	ETC 1002-03
CleanGels 7.5 % 25S	ETC 1001-08
CleanGels 12.5% 25S	ETC 1001-13
CleanGels 15% 25S	ETC 1001-21

Sample buffer:

25 ml rehydration buffer + 500 mg SDS + 80 µl Orange G solution (1%) + 60 µl bromophenol blue solution (1%).
Additional (1ml each): 1% DTT (Dithiothreitol), 4% IAA (Iodoacetamide).

Sample treatment:

Dilute the samples with the sample buffer at least 1 + 1. Dilute as much as possible to reach the upper nonogram region, this gives best results. To control the sample concentration: Take a "Low Molecular Weight" marker (LMW), add 1 ml sample buffer and run at least one lane per gel. After staining procedure, the samples should appear in the same state as this standard lane. Apply 15 -30 µl of each sample, don't leave sample slots unfilled.

After sample dilution add 5% (v/v) DTT-solution to the vials (reduction!) and heat 3 min at 95°C. After the vials are cooled down add 5% (v/v) IOA to the samples (alkylation!).

References

1. Ornstein L. Ann NY Acad Sci. 121 (1964)
2. Laemmli UK. Nature 227 (1970) 680-685