

Pipette with gel-electrophoresis mode improves reliability of electrophoresis results

Dr. Antonio Romaguera, BRAND GMBH + CO KG

Knowing the dispensed-sample quantity is critical for reliable interpretation of electrophoresis bands. The new *Transferpette*[®] *electronic* pipette offers the means to precisely determine and document dispensed volume.

Gel electrophoresis detection limits depend strongly on the protocols employed and the electrophoretic system. Staining with Coomassie Brilliant Blue R-250 is used most frequently to make proteins visible.¹ The detection limit is about 50 – 100 ng per band with this staining method. This detection limit lies at about 5 – 10 ng per band with the much more sensitive silver coloration.² DNA can be visually detected down to about 50 ng/band with ethidium bromide staining.

Factors affecting results

The correlation between the first step of the electrophoresis – gel loading – and the final coloration is often overlooked. Whether a band lies above or below the visual detection limit depends primarily on the concentration and amount of dispensed sample because the intensity of the bands with most staining methods depends mainly on the quantity of macromolecules per band. Accurate determination of the sample quantity dispensed with a micropipette is often difficult because, on the one hand, the amount of aspirated liquid may differ from the pipette setting and, on the other hand, the sample quantity actually delivered to the gel can differ from the nominally set quantity due to casting tolerances or damage of the gel pockets.

GLP guidelines stipulate that all data collected during the execution of a test must be recorded directly, exactly and legibly. The dispensed amount of liquid, which is to be documented exactly according to the GLP guidelines, is decisive for the subsequent analysis of the gels.

Unique control options

The BRAND *Transferpette*[®] *electronic* here provides a unique solution. As with other electronic pipettes, a touch of the pipetting button suffices to pick up the sample. Unlike other pipettes however, the GEL mode of the *Transferpette*[®] *electronic* enables the user to control and document the actual volume aspirated and dispensed during the pipetting operation. For example:

- If the pipetting button is pressed and held during the sample aspiration process, aspiration stops and the display shows the exact amount of sample acquired.
- When the pipetting button is touched again, the pipette dispenses the liquid very slowly at a fixed, uniform speed to avoid the creation of eddies.
- When the pocket in the gel is full and the pipetting button is pressed once again, the motor stops dispensing liquid and the display shows the exact amount of sample dispensed. This is critical information for accurate interpretation of the electrophoresis results if the dispensed volume is less than the aspirated volume. The displayed volume on the

Transferpette[®] *electronic* may then be used in documentation of the test for GLP purposes. In turn, the pattern of the bands can be interpreted correctly since both the concentration and the amount of the dispensed sample are known exactly.

Even small changes of the dispensed volume can make interpretation of the results considerably more difficult. For example, starting with a BSA sample of concentration 5 µg/ml and detection limit of 100 ng/band with Coomassie Brilliant Blue coloration, the results are unambiguously different if only 17 µl are actually dispensed instead of the intended 20 µL.

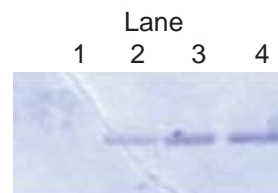


Figure: Detection limit for BSA solution (5 µg/ml) after electrophoresis with Coomassie Brilliant Blue coloration. Lane 1: 17 µl sample; Lane 2: 20 µl sample; Lane 3: 22 µl sample; Lane 4: 25 µl sample.

The pocket with the 17 µl shows no band, because this sample amount lies below the detection limit, whereas the pocket into which 20 µl were dispensed still shows a clearly visible band. These same considerations apply, of course, when testing the purity of proteins and DNA. The new *Transferpette*[®] *electronic* pipette gives the scientist better control over these variables and, thus, the quality of results.

References

1. Fazekas De St. Groth et al. (1963) *Biochim. Biophys. Acta* 71: 377 - 391.
2. Blum, M. et al. (1987) *Electrophoresis* 8: 93 - 99.

Product appearance, catalog numbers, prices, specifications, and technical information are subject to change without notice.