Application Note

Masaryk Memorial Cancer Institute

Title

Comparison of casein and Sophomer F10 as blocking agents for DNA bioassays using magnetic beads and electrode chips

Authors

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Introduction and objectives

Electrochemical (EC) techniques are widely used analytical tools that translate chemical events into measurable electrical signals. Their advantages include low cost, operational simplicity, high sensitivity and selectivity, and compatibility with automation and miniaturization, making them particularly suitable for the development of rapid and user-friendly biosensors.

In our work, we develop electrochemical bioassays for the detection of DNA and RNA biomarkers relevant to cancer research [1–5]. These assays typically employ modified magnetic beads for selective target capture, streptavidin–peroxidase conjugates for enzymatic signal generation, and a blocking agent to minimize nonspecific binding. Casein-based blocking buffers are commonly used for this purpose; however, their animal origin can represent a limitation.

The objective of this study was to evaluate Sophomer F10 as a synthetic, animal-free alternative blocking agent for homogeneous electrochemical bioassays. We compared its performance directly with that of a standard casein blocking buffer in real biological samples (cell lines) during the electrochemical detection of either human cytomegalovirus (at the DNA level) associated with glioblastoma or the BCR::ABL1 fusion (at the RNA level), a key biomarker of chronic myeloid leukemia. Our results demonstrate comparable assay performance, indicating that Sophomer F10 is a promising substitute for casein in electrochemical bioanalytical applications.

References

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Material

- Amplified DNA or RNA using LAMP reaction (loop-mediated isothermal amplification)
- Washing buffer (WB; 5 mM Tris-HCl, pH 7.5; 0.5 mM EDTA; 1 M NaCl)
- 2 M NaCl
- Casein blocking buffer in PBS (CBB; Thermo Fisher Scientific)
- Streptavidin peroxidase polymer (SPP; Thermo Fisher Scientific)
- SophoMer™ F10 1,5% (Sophomer s.r.o.)
- 100 mM phosphate buffer, pH 6.0 (PB)
- H_2O_2 (Sigma-Aldrich)
- Hydroquinone (HQ; Sigma-Aldrich)

Methodology

The DNA (hCMV result) or total RNA (BCR::ABL1 result) isolated from cell lines were amplified using loop-mediated isothermal amplification (LAMP) and subsequently denatured by incubation at 95 °C for 10 min. Carboxylated magnetic beads (MBs) functionalized with sequence-specific capture probes were washed three times with washing buffer (WB). The denatured LAMP product (3.5 µl) was mixed with 7.5 µl of 2 M NaCl and distilled water to a final volume of 25 µl, then incubated with the MBs for 15 min at 40 °C under continuous rotation to enable hybridization.

After incubation, the MBs were washed three times with either casein blocking buffer (CBB) or Sophomer F10 and subsequently incubated for 15 min at room temperature with streptavidin–peroxidase conjugate (SPP) diluted 1,000× in the corresponding blocking buffer. The beads were then washed three times with phosphate buffer (PB) and resuspended in 10 µl of PB.

The resuspended MBs were transferred onto the working electrode and covered with 50 µl of substrate solution containing 50 mM H_2O_2 and 10 mM hydroquinone. Electrochemical detection was performed by amperometry at −0.3 V for 60 s.

Results

Application of Sophomer F10 was evaluated on hCMV (A) and BCR::ABL1 (B) detection bioassay and compared with standard protocol using CBB. In both cases Sophomer showed comparable performance in blocking of negative sample signal with slightly better signal-to-noise ratios as CBB and therefore has potential to be used in electrochemical bioassays.

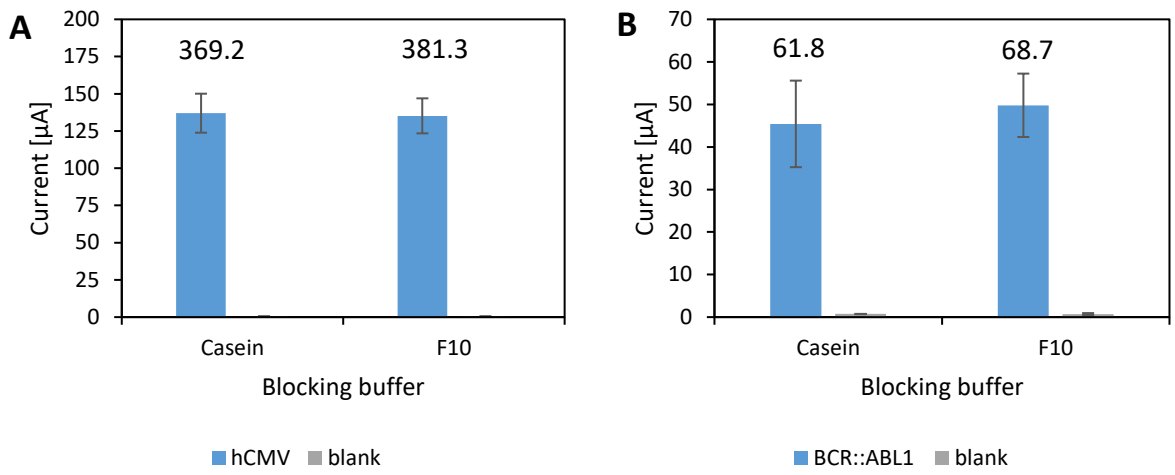

Figure. Comparison of CBB (denoted as Casein) and Sophomer F10 (denoted as F10) in two different bioassays using LAMP products from cell lines positive for: (A) hCMV and (B) BCR::ABL1 fusion transcript (both blue). Blank signals (without DNA, grey) are shown as well. Numbers above columns represent signal-to-noise ratios. Standard deviation is calculated from duplicate measurements.