SDS-PAGE electrophoresis – technique for integrity examination of antibodies

Marija Sterjova[1,2], Predrag Džodić[2], Paulina Apostolova[1], Elena Drakalska[1], Stefanija Ilieva[1], Emilija Janevik-Ivanovska[1]*

[1] University 'Goce Delcev', Faculty of Medical Sciences, str. "Krste Misirkov" No. 10-A, 2000 Stip, R. Macedonia

[2] University of Niš, Faculty of Medicine, Department of Pharmacy, str. dr Zoran Đindić 81, 18000 Niš, Serbia

Review

Monoclonal antibody appears as an important therapeutic agent for the treatment of cancer. Antibodies have shown high complexity in the manner of action and their biological properties. The effectiveness of antibodies can be improved by binding of different cytotoxic drugs, toxins or radioisotopes in order to designed stable immunoconjugates. This allows increasing of specificity and selectivity of drugs and toxins and their targeted accumulation in tumor cells.

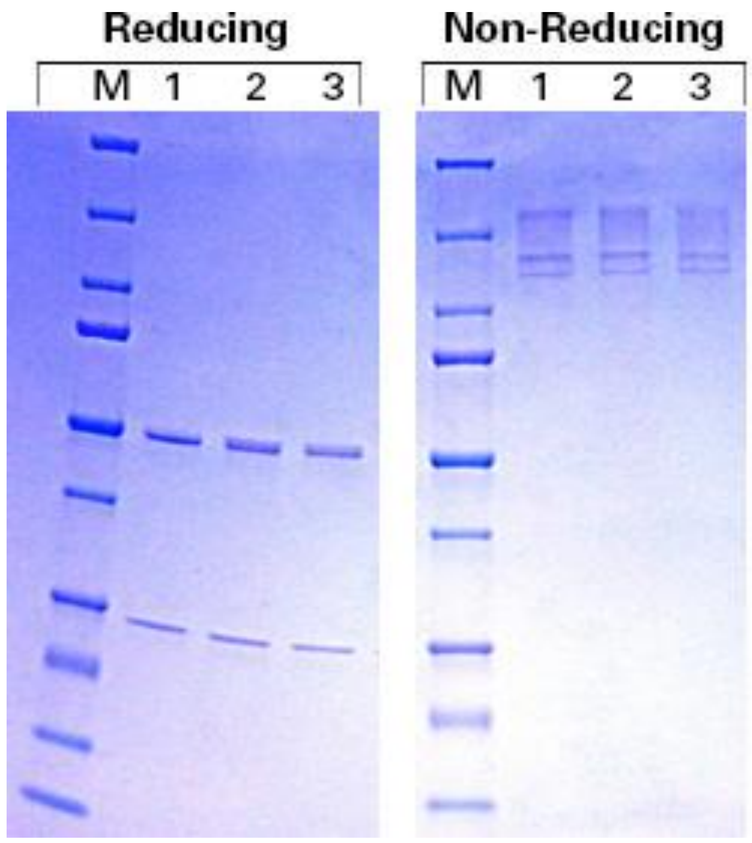

Fig. 1 Reducing and non-reducing SDS-PAGE

After antibody manipulation and conjugation it is important to determine a possible changes in secondary structure of antibodies. The most commonly used technique is SDS-PAGE electrophoresis. As a matrix can be used two polyacrylamide gels with different concentration, stacking gel with lower ratio of acrylamide / bisacrylamide gel for application and separation gel for development of electropherogram.

Many stability studies of antibodies were made using a SDS-PAGE in reducing or non-reducing conditions. In reducing electrophoresis, the 2-mercaptoethanol or dithiothreitol are used as reducing agents for disruption of disulfide bonds, which result with antibody migration as two bands ~50 kDa and ~25 kDa (Mr of heavy and light chain). Under non-reducing conditions only one band at ~150 kDa was observed (Mr of whole antibody).

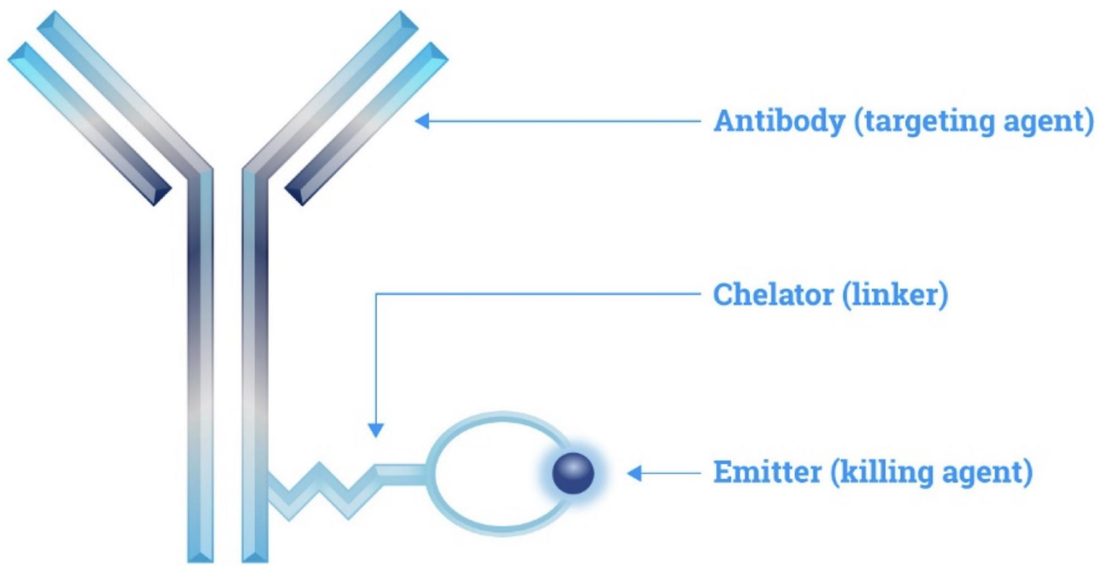

Fig. 2 Conjugated antibody