FAQ - Why do I see too many bands on my Western blot?

Western- or immunoblotting is a commonly employed technique for the detection of protein antigens in complex mixtures. Samples are first separated by SDS-polyacrylamide gel electrophoresis. The separated proteins are then transferred to a membrane (usually nitrocellulose, polyvinyl pyrolidon, or nylon). These membranes are incubated with an antibody specific for the protein of interest which binds to the protein band immobilized on the membrane. The antibody is then visualized with a detection system that is usually based on a secondary protein binding to Ig chains which is linked to a color-yielding reaction.
It is not uncommon that, contrary to the theoretical predictions, several bands are detected. Although it is possible that an antibody is not entirely specific for the protein, other factors may be responsible:

- **Proteolytic breakdown of the antigen.** This is not uncommon, particularly if samples are stored for prolonged time or if proteins or membranes are fractionated after homogenization of the starting tissue. All additional bands are of lower apparent molecular mass than the full-length protein. Particularly susceptible are synapsins and synaptotagmins. Addition of protease inhibitors such as PMSF, pepstatin or leupeptin should be considered.

- **Too much protein per lane or detection system too sensitive.** Overloading of the gel is one of the most common reasons for "ghost bands". Immobilized proteins may provide a concentrated adsorbtive surface to which certain IgG may bind nonspecifically. Similarly, such nonspecific binding may be uncovered when highly sensitive detection systems such as enhanced chemoluminescence are employed. A dilution series of the starting material usually clarifies which of the signals are artefactual.

- **Ineffecient blocking.** A variety of different blocking agents are described in the literature including nonionic detergents and / or proteins. Change of the blocking conditions may remedy the problem.

- **Concentration of antigen too low.** The resolution of SDS-PAGE is limited to 50 - 100 bands. If the relative concentration of the antigen of interest is too low (less than 0.2% of total protein), it may be difficult to detect. For instance, synaptobrevin/VAMP comigrates with histones in cell homogenates which interfere with its detection. Signal enhancement may then lead to the appearance of artificial bands. Enrichment of the antigen by fractionation or by immunoprecipitation should be considered.

Almost all our antibodies were successfully tested for immunoblotting and detect single bands in relatively crude fractions.
Apart from this, several bands are not always the result of suboptimal experimental conditions or cross reactivity. Many proteins have several isoforms, and an antibody may detect more than one of them.