

C L A I M S

1. Method for determining the amount and/or concentration in a sample of at least one ISVD, or of a protein or polypeptide that comprises at least one ISVD, which method
5 comprises the steps of;
- a) contacting the sample with a capturing agent, such that the ISVD, protein or polypeptide is captured by said capturing agent;
 - b) contacting the ISVD, protein or polypeptide captured by the capturing agent with a
10 detection agent, such that said detection agent binds to the ISVD, protein or polypeptide captured by the capturing agent;
 - c) generating a signal corresponding to the amount of detection agent bound to the ISVD, protein or polypeptide captured by the capturing agent,
- in which said method is performed in the presence of a quencher, said quencher being a protein or polypeptide to which pre-existing antibodies that are present in the sample can bind
15 but to which neither the capturing agent nor the detection agent can bind.
2. Method according to claim 1, in which the protein or polypeptide used as the quencher
20 is a protein or polypeptide that is bound by the monoclonal antibody 21-4 produced by the hybridoma ABH0015 deposited with BCCM, Ghent, Belgium under accession number LMBP-9680-CB with an affinity better than 1 micromolar (μM).
3. Method according to claim 2, in which the protein or polypeptide used as the quencher
25 is a protein or polypeptide that is bound by the monoclonal antibody 21-4 produced by the hybridoma ABH0015 deposited with BCCM, Ghent, Belgium under accession number LMBP-9680-CB with an affinity between 1000 and 1 nM.
4. Method according to claim 1 or 2, in which the protein or polypeptide used as the
30 quencher is a protein or polypeptide that is bound by the intended capturing agent and the intended detection agent with an affinity of less/worse than 3 micromolar.

5. Method according to claim 4, in which the protein or polypeptide used as the quencher is a protein or polypeptide that is bound by the intended capturing agent and the intended detection agent with an affinity of less than 10 micromolar.

5 6. Method according to claim 4, in which the protein or polypeptide used as the quencher is a protein or polypeptide that is bound by the intended capturing agent and the intended detection agent with an affinity of less than 50 micromolar.

10 7. Method according to claim 4, in which the protein or polypeptide used as the quencher is a protein or polypeptide that is bound by the intended capturing agent and the intended detection agent with an affinity of worse than 100 micromolar.

15 8. Method according to claim 1 or 2, in which the quencher is a monovalent immunoglobulin single variable domain or a fusion protein or construct comprising not more than five immunoglobulin single variable domain(s).

20 9. Method according to claim 8, in which the quencher is a monovalent immunoglobulin single variable domain or a fusion protein or construct comprising four, three, two or one immunoglobulin single variable domain(s).

10. Method according to claim 8, in which at least one of the immunoglobulin single variable domains present in the quencher has an exposed C-terminal end.

25 11. Method according to claim 10, in which the at least one of immunoglobulin single variable domain that has an exposed C-terminal end has the sequence VTVSS (SEQ ID NO:1) at its C-terminal end.

12. Method according to claim 8, in which at least the quencher has an immunoglobulin single variable domain at its C-terminal end.

30 13. Method according to claim 12, in which the immunoglobulin single variable domain at the C-terminal end of the quencher (and, by extension, the entire quencher) has the sequence VTVSS (SEQ ID NO:1) at its C-terminal end.