

C L A I M S

1. Method for determining the amount and/or concentration in a sample of at least
 5 one immunoglobulin single variable domain (ISVD), or of a protein or polypeptide that
 comprises at least one ISVD, which method comprises the steps of;

 - a) contacting the sample with a capturing agent, such that the at least one ISVD, protein or
 polypeptide is captured by said capturing agent;
 - b) contacting the at least one ISVD, protein or polypeptide captured by the capturing agent
 10 with a 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 at least
 one 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
 15 protein or polypeptide to which pre-existing antibodies against an exposed C-terminal end of
 the at least one ISVD or an exposed C-terminal end of the at least one ISVD of the protein or
 polypeptide can bind but to which the capturing agent and the detection agent bind with an
 affinity (expressed as K_D) of worse than 3 micromolar (μM), i.e. $K_D > 3 \mu\text{M}$.
- 20 2. Method according to claim 1, in which the protein or polypeptide used as the quencher
 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 (expressed as K_D) better than 1 micromolar (μM), i.e. $K_D <$
 1 μM .
- 25 3. Method according to claim 2, in which the protein or polypeptide used as the quencher
 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 (expressed as K_D) between 1000 and 1 nanomolar (nM), i.e.
 30 1 nM $< K_D < 1000$ nM.
4. Method according to any one of claims 1 to 3, 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 (expressed as K_D) of worse than 10 micromolar (μM), i.e. $K_D > 10 \mu\text{M}$.

5. Method according to any one of claims 1 to 3, in which the protein or polypeptide
5 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 (expressed as K_D) of worse than 50 micromolar (μM), i.e. $K_D > 50 \mu\text{M}$.

6. Method according to any one of claims 1 to 3, in which the protein or polypeptide
10 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 (expressed as K_D) of worse than 100 micromolar (μM), i.e. $K_D > 100 \mu\text{M}$.

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

8. Method according to claim 7, in which the quencher is a monovalent immunoglobulin
single variable domain or a fusion protein or construct comprising four, three, two or one
20 immunoglobulin single variable domain(s).

9. Method according to claim 7, in which at least one of the immunoglobulin single
variable domains present in the quencher has an exposed C-terminal end.

10. Method according to claim 9, in which the at least one of immunoglobulin single
variable domain present in the quencher that has an exposed C-terminal end has the sequence
VTVSS (SEQ ID NO:1) at its C-terminal end.

11. Method according to claim 7, in which at least the quencher has an immunoglobulin
30 single variable domain at its C-terminal end.

12. Method according to claim 11, 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.