Using the immunizing peptide to block primary-derived signal

The immunizing peptide can be used to verify the specificity of the primary antibody. For this purpose the appropriate diluted antibody is split and one half is pre-adsorbed to the immunizing peptide while the other half is kept in parallel at the same dilution but without the peptide. One blot or tissue slide is then exposed to the un-exposed primary while an identical other one is exposed to the pre-adsorbed antibody.

The peptide is delivered as a 100ug pellet. When peptides are reconstituted in 200ul water, the concentration would be 0.5mg/ml, which is equal to the concentration of the antibodies. To start a blocking experiment, the best ratio would be 1:1 (which means molar excess of peptides relative to antibodies when identical volumes are mixed).

- Reconstitute the 100ug pellet in 200ul water in order to generate 0.5mg/ml peptide solution.
- Mix equal volumes of antibody (comes as 0.5ug IgG/ml) and peptide at the required primary dilution in blocking buffer
- In parallel dilute identically just the primary in blocking buffer.
- Leave both at ambient temp for 1h.
- Have two identical blots/slides ready in blocking buffer
- Replace the blocking buffer with the antibody, one blot/slide without the peptide.
- Incubate for 1h at ambient temp
- Follow labelling protocol

Blocking:

Please mix equal volumes of peptide and antibody at the required dilution and leave at ambient temperature. Best is to have two identical blots, to be incubated with equal amount of antibodies, but one with the antibodies pre-adsorbed to the peptide for 20min. Then incubate and develop in parallel the two blots.