I. Protein Properties

1. General
   a) What is the molecular weight of the protein in Dalton? (Da)
   b) Structure of the protein? □ globular □ planar □ others, please specify: ____________________
   c) pI -value : _______

2. Structure
   a) How many sub-units does the protein comprise? _____________
   b) In which way are these sub-units linked to each other?
      □ covalently □ hydrogen bonding □ disulfide bridges □ others, please specify: _______
   c) What is the amino acid sequence?
      Please attach sequence as a separate document or indicate the access ion number of the public data base where the sequence has been published :
      ____________________________________________
   d) Is there a modification/blockage at the N-terminal amino acid of the protein?
      □ yes □ no □ don’t know
   e) How many cysteine residues does the protein contain, how many of them are bound within disulfide bridges?
      ____________________________________________
   f) Is there any information about single disulfide bridges that maybe reduced without losing the functionality/bioactivity of the protein?
      □ yes □ no
      if yes, please specify___________________

3. Function
   a) What is the biologic function of the protein in-vivo?
      □ enzyme □ structural protein □ antibody □ growth factor □ hormone □ others, please specify below:
      ____________________________________________
   b) Does the protein need any co-factors for action?
      □ yes □ no
      if yes, please specify: _______
   c) Which functional groups or sections are essential for biological activity of the protein?
      ____________________________________________
   d) Is there an easy assay for the determination of in-vitro activity?
      □ yes □ no
      if yes, please specify
      ____________________________________________
      ____________________________________________
      ____________________________________________
II. Delivery of protein sample

1. Which quantity of protein in mg would be available for PEGylation experiments?
   ___________mg

2. What is the purity of the protein (electrophoresis)?
   ______________________________________

3. In which formulation will the protein be provided to celares GmbH?
   ☐ as a solution in water  ☐ as a solution in buffer
   ☐ as lyophilised powder, without additives  ☐ as lyophilised powder, with additives

4. What would be the ideal storage and buffer conditions for the protein:
   - Temperature __________________________°C
   - pH-value ______________________________
   - Ionic strength of buffer __________________________mM
   - Additives of stabilizers ______________________________

5. Which purification procedure do you usually use for the protein?
   ______________________________________________________
   ______________________________________________________
   ______________________________________________________

III. Goal of a PEGylation

1. Which properties of the protein should be modified by PEGylation?
   ☐ Immunogenicity ☐ Protease stability ☐ renal excretion ☐ Solubility in aqueous solutions ☐ others, please specify:______________________________

2. Have you already identified a functional group or amino acid that should be PEGylated?
   ☐ yes ☐ no  if yes, please specify:______________________________

3. Are there any special requirements with respect to Degree of PEGylation
   ☐ no ☐ yes, please specify______________________________
   Site of PEGylation ☐ no ☐ yes, please specify______________________________

IV. Experience with regard to PEGylation

1. Have you already performed any PEGylation studies? ☐ yes ☐ no
   - If yes, please answer the following questions –

2. Target group for PEGylation
   ☐ amine ☐ thiol ☐ hydroxyl ☐ carboxyl ☐ others, please specify: __________

3. Activation chemistry of the PEG reagent used for PEGylation:
   ________________________________

4. Molecular weight of the PEG reagent _____________Dalton

5. Who used to be the supplier of the PEG, Order No., and Lot-No etc. if applicable?
   ______________________________________________________
6. What were the PEGylation conditions used so far?
   a) Buffer: ____________________________________________
   b) pH-value: __________________________________________
   c) Temperature: _____________________________ °C
   d) Period: ________________________________ h
   e) Protein conc.: ______________________________ mg/mL
   f) What was the ratio of PEG/protein?: ___________________________ equivalents

7. What was the resulting degree of PEGylation? ________% (compared with maximal theoretical PEGylation)

8. What was the residual biological activity of the protein after PEGylation in % activity of the native protein? _______________

9. Did you achieve any improvement with regard to the target parameters by PEGylation?
   □ yes, please specify: __________________________________________
   □ no

Thank you very much