1) Cells before

a) Essential information about the donor
   i) Species and strain
      Species
      Strain (if applicable)
   ii) Characteristics of the organism
      Health
      Age
      Treatment/Environment
      Individual identifier number
      Source of purchase (if applicable)

b) Source of cell material
   Organ, tissue, fluid or blood product
      Source (if applicable)
      Quantity (volume, size or weight)
      Anti-coagulant (if applicable)

c) Cell separation process
   Cell separation method
   Equipment used
   Tissue conditions between tissue retrieval and cell separation
      Duration
      Temperature
      Fluid
      Container
   Purity of the cells after the separation process
      Methodology

d) Phenotype
   i) Morphology
      Shape and appearance of cells
   ii) Cell surface and intracellular markers
      Molecules measured (using CD names)
      Methodology
      Stimulus and time of stimulation (if applicable)
iii) Secreted molecules
Molecules measured
Methodology
Stimulus and time of stimulation (if applicable)

e) Cell numbers
i) Absolute cell number
Total number of cells at the end of the isolation process
Methodology

ii) Viability
Percentage of viable cells
Methodology

2. Differentiation and induction of tolerogenicity (diff/tol)

a) Pre-culture conditions
Storage time
Storage conditions
If fresh
- Fluid
- Container
- Temperature
If cryopreserved
- Freezing/thawing process
- Freezing medium
- Cell recovery & viability after thawing

b) Culture conditions
i) Cell number
The total number of cells put into culture

ii) Cell concentration
The number of cells per ml of medium at start of culture

iii) Culture medium
- Type(s) of medium
- Source(s)
- Additives (excluding diff/tol agents)
- Source(s)
- Refreshment of the medium

iv) Culture container
- Type of container
- Size
- Manufacturer
- Cell culture volume per container or well
- Total number of containers or wells
3. Culture environment

- Temperature and CO2 concentration
- Use of pre-warmed medium
- Equipment

c) Differentiation/induction of tolerogenicity (diff/tol) protocol

- Protocol
- Name of cytokine(s) or other agent(s) used
  - Source
  - Concentration
  - Time-point(s) added to cell culture
- Total length of the culture period

d) Antigen

- Name
- Source
- Concentration
- Time point(s) added to culture
- Carrier (if applicable)

e) Storage

- Storage time
- Storage conditions
  - If fresh
    - Fluid
    - Container
    - Temperature
  - If cryopreserved
    - Freezing/thawing process
    - Freezing medium
    - Cell recovery & viability after thawing
    - Time point at which cells are stored if different to the end of the culture process

3. Cells after

a) Phenotype

i) Morphology

- Shape and appearance of cells

ii) Cell surface and intracellular markers

- Molecules measured (using CD names)
  - Methodology
- Stimulus and time of stimulation (if applicable)
iii) Secreted molecules
Molecules measured
Methodology
Stimulus and time of stimulation (if applicable)

b) Cell behaviour
Behaviour of cells in a functional assay

c) Cell numbers
i) Absolute cell number
Total number of cells at the end of the isolation process
Methodology

ii) Viability
Percentage of viable cells
Methodology

4. About the protocol

a) Regulatory authority
External authority that approved the protocol
Does protocol follow GMP?

b) Purpose
The reason for manufacturing the cells

c) Relationship between the source organism for the cells and the target organism
Allogeneic/Autologous/ Xenogeneic/Syngeneic

d) Contact details
Name(s) of the corresponding author(s)
Contact details of the corresponding author(s)