Sedimentation velocity is aimed to obtain the sedimentation coefficient distribution of the specie/s present in the sample and their number and concentration. Sedimentation velocity assays usually take one day and one or two extra days for the data analysis. The volumes required are 400 microliters per condition (concentration) and at least the same volume of buffer (used by the machine as reference).

Sedimentation equilibrium assays are aimed to get the molecular weight average of the macromolecular complex. Sedimentation equilibrium assays take 48-72 hours, hence the sample must be stable at least for this time period. The volumes required for equilibrium assays are 100 microliters per concentration, and at least the same for the buffer (used by the machine as reference).

The issues to take into account are:

1- Generally, the concentration range should be as wide as possible, with the constraint not to exceed 2 mg/mL of protein (to prevent non-ideal sedimentation), and to remain well within the detection limits (0.15 OD-1.3 OD) at the wavelength chosen (230-300 nm).
2- Regarding the buffer composition, it should be avoided the use of glycerol, although we can accept as much as a 5%, and components that interfere with the optical detection, such as beta-mercaptoethanol, DTT, etc, although we can also accept as much as 0.1 mM of these reducing agents and preferably TCEP, which is more stable.
3- The protein purity should be 95%, and it is recommended to perform size-exclusion chromatography as the last preparative step.
4- Maximum number of samples per run is seven (buffer is not considered as a sample).