



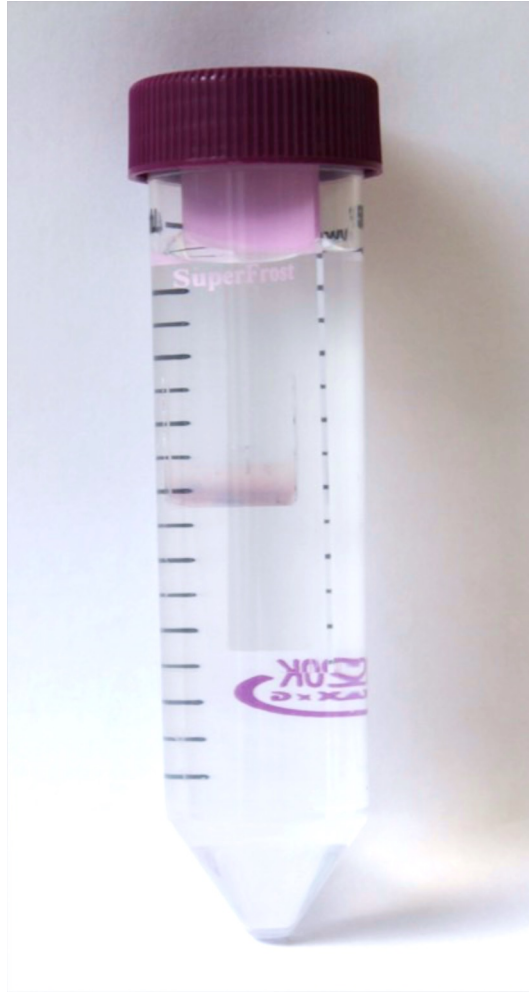
Cline Nano Surfaces & Cline Nano Gradients



The Guide

How to handle & modify the
Cline Nano Surface & Cline Nano Gradient

Length of protocol: 3h + Over night



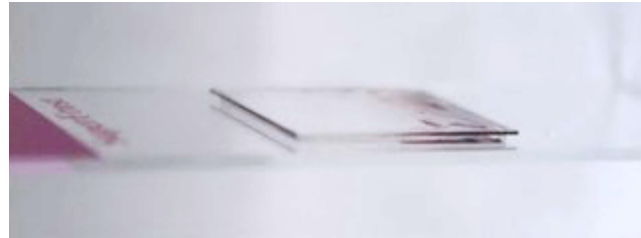
The Container

The Cline Nano Surface or Cline Nano Gradient is shipped in a water filled tube.

Step 1

The Cline Nano Surface or Cline Nano Gradient is attached to a microscope slide with a small silicone cushion

Make sure that the surface of the glass pointing outwards from the cushion, called up, is wet and untouched at all times.



➔ Carefully turn the tube while inspecting the surface to make sure which side is up!

Step 2

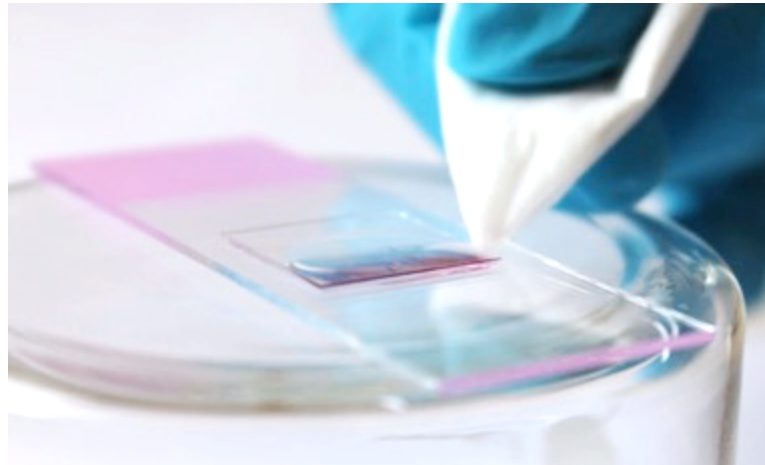


- ➔ Open the lid
- ➔ Firmly grip the microscope slide by its end
- ➔ Gently squeeze the tube to release the slide

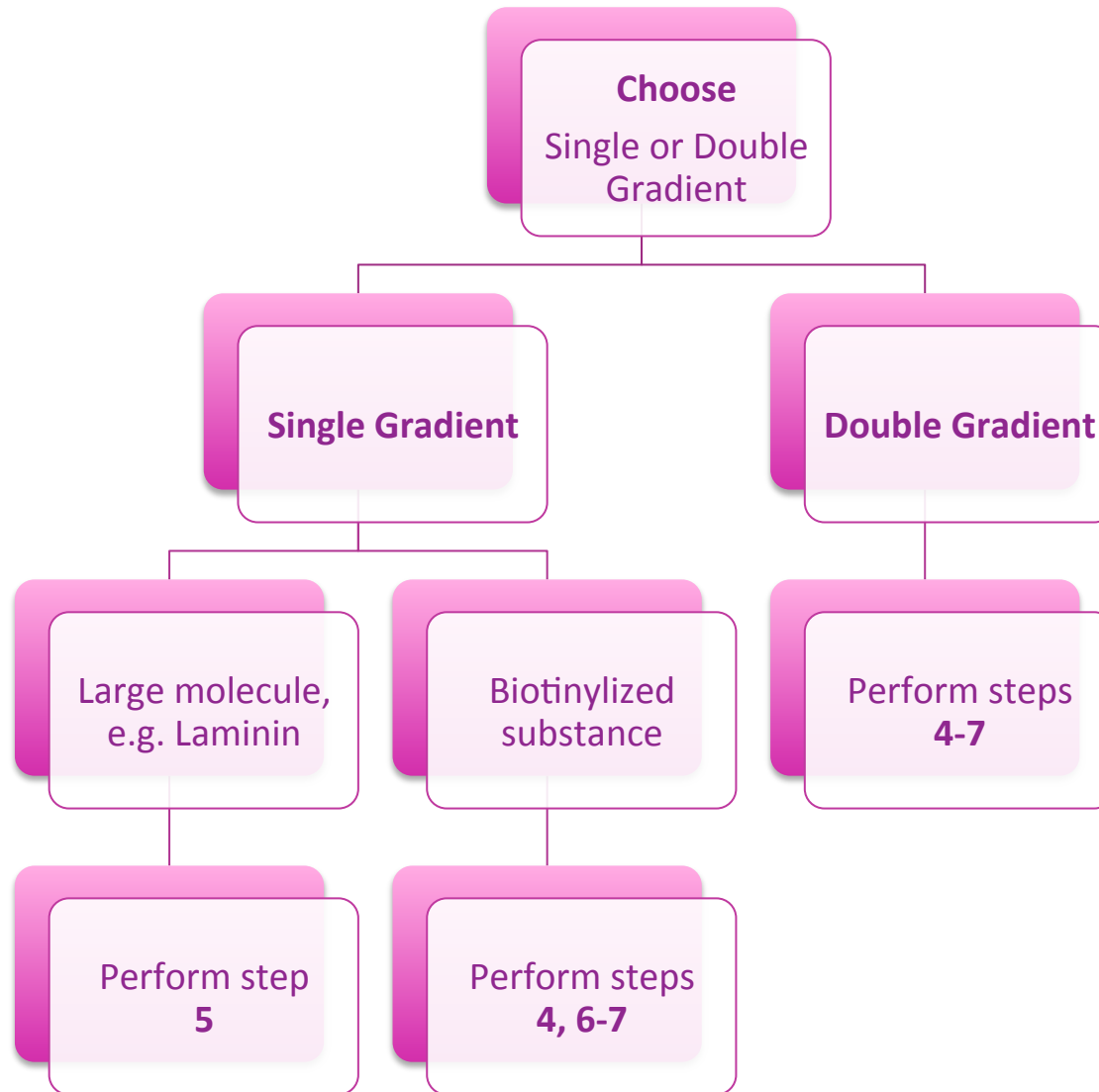
Step 3

- ➔ Place the microscope slide on a flat clean surface (preferably inside a laminar flow hood)
- ➔ Carefully remove excess liquid without touching the surface, directly before adding solutions (see step 4-7)

OBS: It is imperative that the surface never dries out!



Instructions for Step 4-7



Step 4

Preparing the nanoparticle gradient

- ➔ Add 540ul water to Vial 1 (Linker 1)
(enough for 2 surfaces)
- ➔ Dropwise place ca. 300ul of Linker 1 on the surface
- ➔ Incubate for 30 min in room temp
- ➔ Slowly rinse with water

Step 5

Modifying space between nanoparticles – gradient of e.g. Laminin

- ➔ Dropwise place ca. 300ul of e.g. Laminin (20uM in PBS) on the surface. Incubate for 1.5 h in room temp.
- ➔ Slowly rinse with PBS.

Tip: Protect the incubation from contamination and evaporation by covering the surfaces with a lid or a closed environment

Step 6

Adding a linker on the nanoparticles

- ➔ Dissolve the contents of Vial 2 (Linker 2) in 1.98ml PBS (enough for ca. 6 surfaces)
- ➔ Dropwise place ca. 300ul Linker 2 on the surface
- ➔ Incubate for 20 min in room temp
- ➔ Slowly rinse with PBS.

Step 7

Adding biotinylated substance – Gradient of biotinylated substance on nanoparticles

- ➔ Biotinylate your biomolecule
- ➔ Dropwise place ca. 300ul biotinylated substance (40uM in PBS) on the surface
- ➔ Incubate in the fridge over night
- ➔ Slowly rinse with PBS

The surface is now ready for use!

Optional Step

Removing and transferring the Cline Nano Surface or Cline Nano Gradient

- ➔ Carefully grab the glass surface in one un-colored corner.
- ➔ Gently pull the glass surface on the silicone cushion upwards slowly to release it from the microscope slide
- ➔ If necessary; use a second pair of tweezers to remove the cushion from the back of the glass
- ➔ Keep track of which side of the glass surface is up!
- ➔ Place the surface in your cell culture well (with or without silicone cushion)

