

STAINING PROTOCOL

Actin & DAPI

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for staining of F-actin and DNA in whole cell laden constructs with ActinGreen™ 488 ReadyProbe and NucBlue™ Fixed Cell ReadyProbes. This protocol is performed on fixed cell laden constructs, Follow *Fixation Protocol* before starting this protocol. This protocol has been validated for a wide range of 3D bioprinted cell laden constructs.

Materials needed

- Fixed cell laden constructs, stored in Hank's Balanced Salt Solution (HBSS+/+).
- HBSS+/+
- ActinGreen™ 488 ReadyProbe, ThermoFisher Scientific, Cat.: R37110
- NucBlue™ Fixed Cell ReadyProbes, ThermoFisher Scientific, Cat.: R37110

Protocol

This protocol can be performed non-sterile, always protect the samples from light when stained.

1. Actin staining

MATERIAL

Fixed cell laden constructs

HBSS+/-

ActinGreen

DESCRIPTION

- Remove the HBSS+/- that the constructs are stored in. If stored in ethanol, wash with HBSS+/- before adding the dye.
- Estimate the amount of dye needed to cover the cell laden constructs. Staining can be performed for example in well plates or Eppendorf tubes with one or several constructs in each tube/well.
- Prepare the dye:
 - Alternative 1: 2 drops of ActinGreen/mL HBSS+/-, incubate samples for 2-4 h.
 - Alternative 2: 1 drop of ActinGreen/mL HBSS+/-, incubate overnight.
- Submerge the constructs in the dye and incubate according to the chosen alternative. Protect the constructs from light with foil during the incubation.

2. DAPI staining

MATERIAL

HBSS+/-

NucBlue

DESCRIPTION

- Remove ActinGreen dye from the constructs.
- Use the same estimated volumes from Step 1.
- Prepare the dye: 2 drops of NucBlue/mL HBSS+/-.
- Submerge the constructs in the dye and incubate samples for 1 h. Protect the constructs from light with foil during the incubation.

3. Washing

MATERIAL

HBSS+/-

DESCRIPTION

- Wash the constructs 3 x 10 min with HBSS+/- at room temperature.
- Image directly or store the samples in the dark at 4°C, samples stay stained until the fluorophores bleach.