

Protocol for BioFix Scaffolds 35 & 175

1. Sample preparation

- a. Prepare the sample by thoroughly mixing the BioFix Buffer with your reagent of choice.

We recommend mixing your reagent of choice with BioFix Buffer at a 1:1 ratio, with a maximum total volume per scaffold type listed in the table below.

We do not recommend exceeding 1 µg of reagent per 1 µl of BioFix Buffer.

We also recommend adding a carrier protein such as BSA at 100 mg/mL to reduce protein loss to surfaces. This is especially important when working with protein concentrations of < 100 µg/mL. Make sure to mix gently until clear - do not use if the mixture is foamy or cloudy.

BioFix Scaffold Type	Column Size	Insertion Tool Size	Recommended Volume (µl) (reagent + BioFix Buffer)	Max mass of reagent (µg)	Cycle Name	Cycle Duration
Scaffold 35	15mL	IT35	35	17.5	BF35	90 minutes
Scaffold 175	50mL	IT175	175	87.5	BF175	90 minutes

2. Stabilizer Initialization

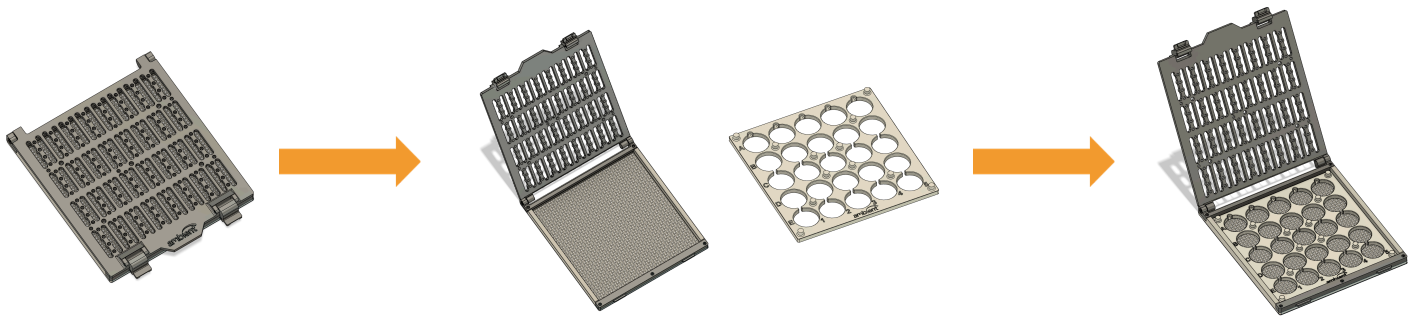
- a. Press the white button to turn on the Stabilizer.
- b. Insert the SD card and press the green button to confirm. Use the red button to visualize different cycle options and use the green button to select a cycle. Once the cycle is confirmed the Stabilizer will start to initialize. A series of beeps will indicate the chamber is ready.

3. Scaffold Preparation

- a. Open the BioFix tray (included in the equipment box and pictured below) by unlatching the 2 locks at the front. .



- b. Place the appropriate white tray insert into the BioFix tray. These are included in the equipment box. (Pictured is the BioFix 175 set up).
- c. Place one scaffold into each designated area.
- d. Pipette BioFix Buffer/reagent mixture onto each scaffold.
- e. Close the lid on the tray and secure it by locking the 2 latches on the front.

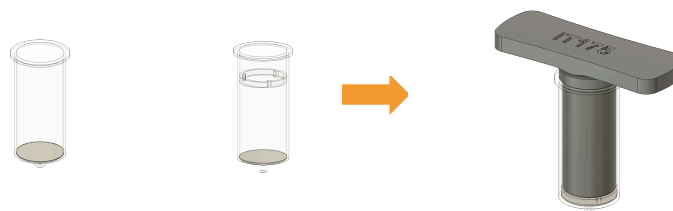


4. Run the Stabilization Cycle

- a. Confirm the chamber is ready by checking the screen says 'Start Cycle?'
- b. Place the tray into the chamber and close the chamber door, tightening the knobs clockwise.
- c. Press the green button to begin the cycle.

5. Column Assembly & Storage

- a. Place the scaffold into the designated stabilization column (supplied and pictured below).
- b. Insert the capture ring into the column
- c. Using the appropriate insertion tool, push the capture ring all the way down to secure the scaffold. The column can be placed into the corresponding 15 mL or 50 mL conical tube and capped.
 - i. If you are using the samples immediately go to step 6.



- d. Place the assembled column/tube into the storage bag (with 2 desiccants).
- e. Zip seal the mylar bag shut. Clip seal using the provided closures.
- f. The sample can now be stored at ambient temperature.

6. Sample Reconstitution

- a. The stabilized sample can be eluted from the column with desired eluent. Refer to the table below for the volume of eluent that can be used.

BioFix Scaffold Type	Minimum Elution Volume (mL)	Maximum Elution Volume (mL)
35	0.5	4
175	1	20

- b. Incubate at room temperature for ≥ 30 seconds.
- c. Centrifuge the spin column-tube assembly at $\geq 300 \times g$ for 2 minutes.
- d. The flow-through now contains your reagent and is ready for use.

