

## InstiGRO™ HEK Supplement – 2mL Sample

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**Product Code:** RS-1302

### Description

InstiGRO™ HEK is an animal component free cell culture supplement designed to enhance growth and improve the cloning efficiency of suspension HEK cells in liquid or semi-solid medium. InstiGRO HEK is supplied as a 20x concentrated stock solution which should be diluted to a final concentration of 1-1.5x depending on the application.

### Intended Use

FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL, DIAGNOSTIC, OR THERAPEUTIC USES.

### Storage Conditions

Store at 2-8°C. Do not freeze.



### Shelf-Life Information

InstiGRO HEK is stable for 18 months from the date of manufacture (see Certificate of Analysis on our website - <https://www.aicompanies.com/support/certificates-of-analysis/>).

### Directions for Use

You have been provided with a 2mL sample of InstiGRO HEK to trial for single cell cloning, please read the instructions below carefully before you begin. It is important to set up the experiment below using your standard cloning conditions in parallel. At 37°C precipitate may sometimes be observed; however, this will not affect the reagents performance. For applications where optical clarity is important, filter the product (0.2µm) prior to use.

1. Gently invert the vial of InstiGRO HEK several times (do not shake) prior to use.
2. Count the cell suspension and ensure that cell viability is ≥95%. Dilute the cell suspension to 2x10<sup>3</sup> cells/mL and transfer 0.95mL of the suspension to a 1.5mL Eppendorf tube.
3. Add 50µL of the InstiGRO HEK stock solution to the 1.5mL Eppendorf tube containing the diluted cell suspension for a final concentration of 1x InstiGRO HEK in a total volume of 1 mL. This step will prime the cells ahead of performing single cell cloning.
4. Incubate the diluted cell suspension with instiGRO HEK for 4-24 hours prior to single cell seeding at 37°C.
5. For single cell cloning, a total of 3 x 96 well flat bottom plates should be seeded via limiting dilution (0.5 cells/well). Filter the remaining InstiGRO HEK stock solution using a 0.2µm filter before adding to the cell culture medium. Add 1.75mL of the InstiGRO HEK stock solution to 33.25mL of media to prepare 35mL of 1x InstiGRO HEK cloning medium.
6. Add 87.5µL of primed cell suspension to 34.13mL of InstiGRO HEK cloning medium for a final cell concentration of 5.1cells/mL.
7. Using a multichannel pipette, dispense 100µL of suspension per well of each 96 well plates. Once complete, place the plates into the incubator and allow cells to incubate for 14 days.
8. After 14 days, assess the clonal outgrowth achieved with InstiGRO HEK cloning medium.