

# Optimizing Small Scale Devices for Antibody Screening

The Thomson Optimum **Growth™** Family of products is expanding to include culturing and purifying in 96 & 24 well plate format. The 96 & 24 well plates offer a scalable option that

work in conjunction with 125mL & 250mL Optimum **Growth™** Flasks and Ultra **Yield™** Flasks. Here we present data optimizing growth conditions and filtration options.

## Optimizing HEK293 Culture in 24 Well Plates

### Materials and Methods

Three different 24 well plates (Axygen V-Bottom, Thomson V-Bottom and Thomson Round-Bottom) and six different volumes (2mL, 2.5mL, 3mL, 3.5mL, 4mL, 4.5mL) were evaluated for expression levels of Rabbit IgG in HEK-293 cells. The plates were shaken @ 250rpm, with a 50mm throw. They were transfected using two in-house plasmids that express Rabbit IgG. Expression levels were evaluated at the different volumes to see if the volume could be increased without impacting yield.

### Results

The expression levels from the HEK-293 transfections from the Thomson 24 Well, V-bottom plates, p/n 931571 was consistent in all volumes for both Plasmid 1 and Plasmid 2. This shows that one can easily **scale up from 2mL to 4.5mL to increase the recombinant protein yield**. Purification of the IgG's was easier with the Thomson 24 Well, Round-Bottom Plates, p/n 931568. The beads appeared to stick in the corners of both the 24 Well V-Bottom Plates. For results see fig. 1

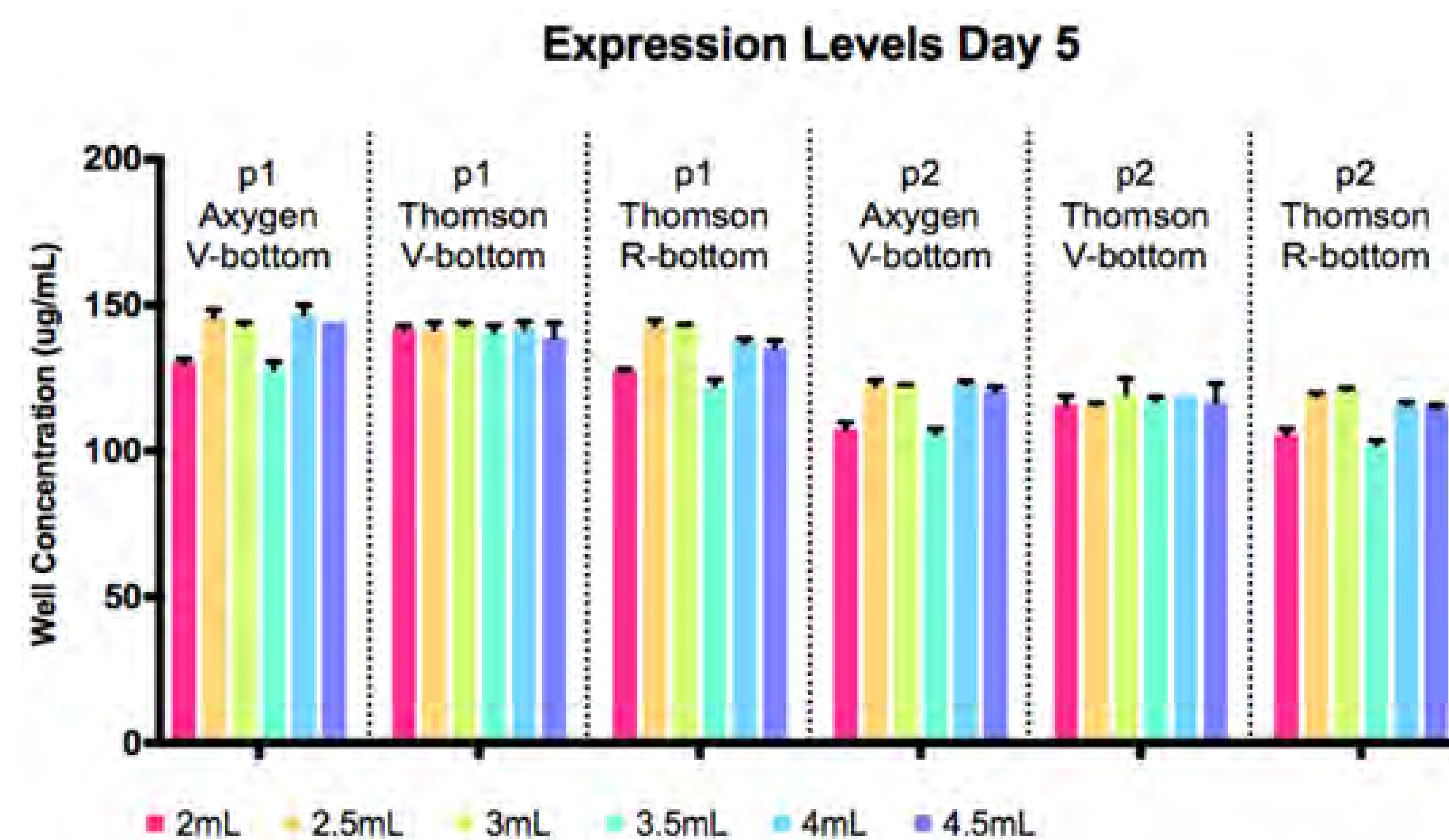


Fig 1. Expression Levels of HEK-293 cells after 5 day growth for 2 plasmids.

## Optimizing Clarification Step & Purification Step by using Thomson 24 Well Filter Plates

Cells grown in Thomson 24 Well Plates may be harvested using the Thomson Rapid Clear: 24 Well 0.2µm Filter Plate, p/n 921546 (Clarification Filter Plate), 24 Well 25µm (Purification Filter Plate) p/n 921550 and a Thomson 24 Well Collection Plate, p/n 931565-G-1X. Alternatively culture can be transferred to a Filter Plate from either a bioreactor, spin-tube, or Optimum **Growth™** Flask.

### Clarification:

- 1) Sterile filtration of cellular material can be processed using the Rapid Clear 24 Well 0.2µm Filter Plate, p/n 921546 .
  - a) Filling From Flask just pour into Filter Plate with collection plate underneath.
  - b) Remove from Bioreactor, and spin soup down at 2000g for 5 minutes, and then pour 4mL into Filter Plate with collection plate underneath.
- 2) Filtration Step 3 different methods:
  - a) Centrifugation 2500rpm for 10-15 minutes or Vacuum manifold, Thomson p/n 981803, @ 20psi , or use positive pressure @ 20psi.

### Purification:

- 1) Cellular material is loaded into the filter plate either manually or using a robotic liquid handling system. The appropriate resin for purification is added after the cellular material to prevent the resin from drying. The 24 Well Filter Plates are stacked with collection plate underneath.
- 2) Filtration Step 3 different methods:
  - a) Centrifugation 2500rpm for 10-15 minutes or Vacuum manifold, Thomson p/n 981803, @ 20psi , or use positive pressure @ 20psi
- 3) The final purified material is eluted off of the resin beads into a second sterile collection plate.



Fig 1. 24 Well Round Bottom Plate, p/n 931565-G-1X



Fig 2. 24 Well Filter Plate, p/n 921546

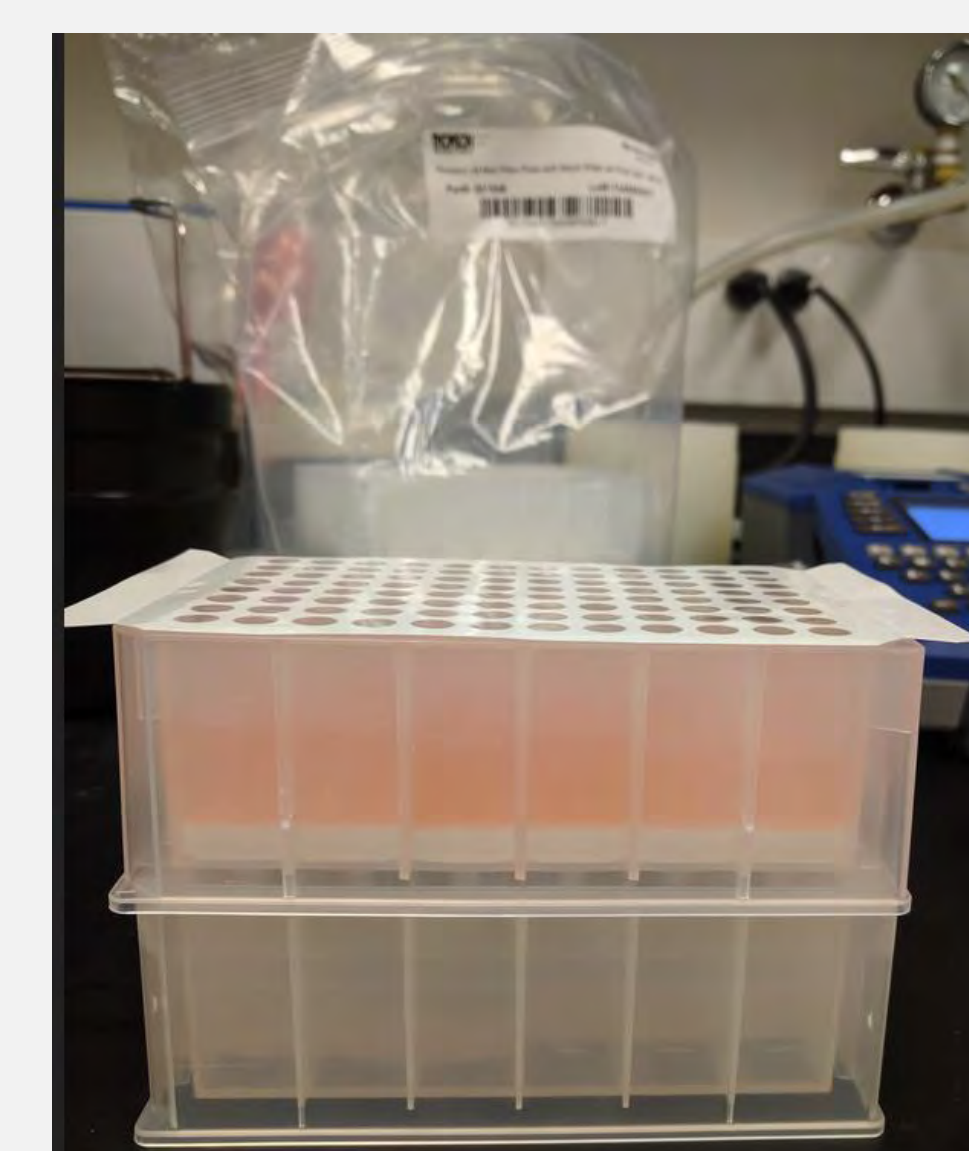


Fig 3. Culture to be filtered in a 24 well filter plate into a 24 well plate



Fig 4. Media and Secreted Antibody & protein ready for analysis

Please stop by the booth for additional information and 96 well cultures protocol.

## Transient Protein Expression in HEK-293 Cells Grown in Thomson 250mL Optimum Growth™ Flasks

Transient protein expression levels were compared in HEK 293 cells between Corning® 250mL flasks and Thomson 250mL Optimum **Growth™** Flasks 50mL of working volume in a Corning® 250mL flask was compared to 100mL, 140mL, and 150mL of working volume in Thomson 250mL Optimum **Growth™** Flasks for titer, VCD, and viability. All flasks were shaken at 150rpm in a 1" orbit and fed on day 3 with glucose. The Thomson Optimum **Growth™** Flask had the highest final titer and was 25% higher than the titer of the 250ml Corning® Flask. Less than 10mg of protein was expressed in the 250mL Corning® flask and 40mg of protein was expressed in the Thomson 250mL Optimum **Growth™** The VCD and cell viability of the Thomson 250mL Optimum **Growth™** Flask with 140mL of working volume were comparable to the Corning® 250mL flask with 50mL of working volume.



Fig 3. Optimum **Growth™** 250mL Flasks in 8 Position Carrier (p/n 1212905) and in Infors Shaker

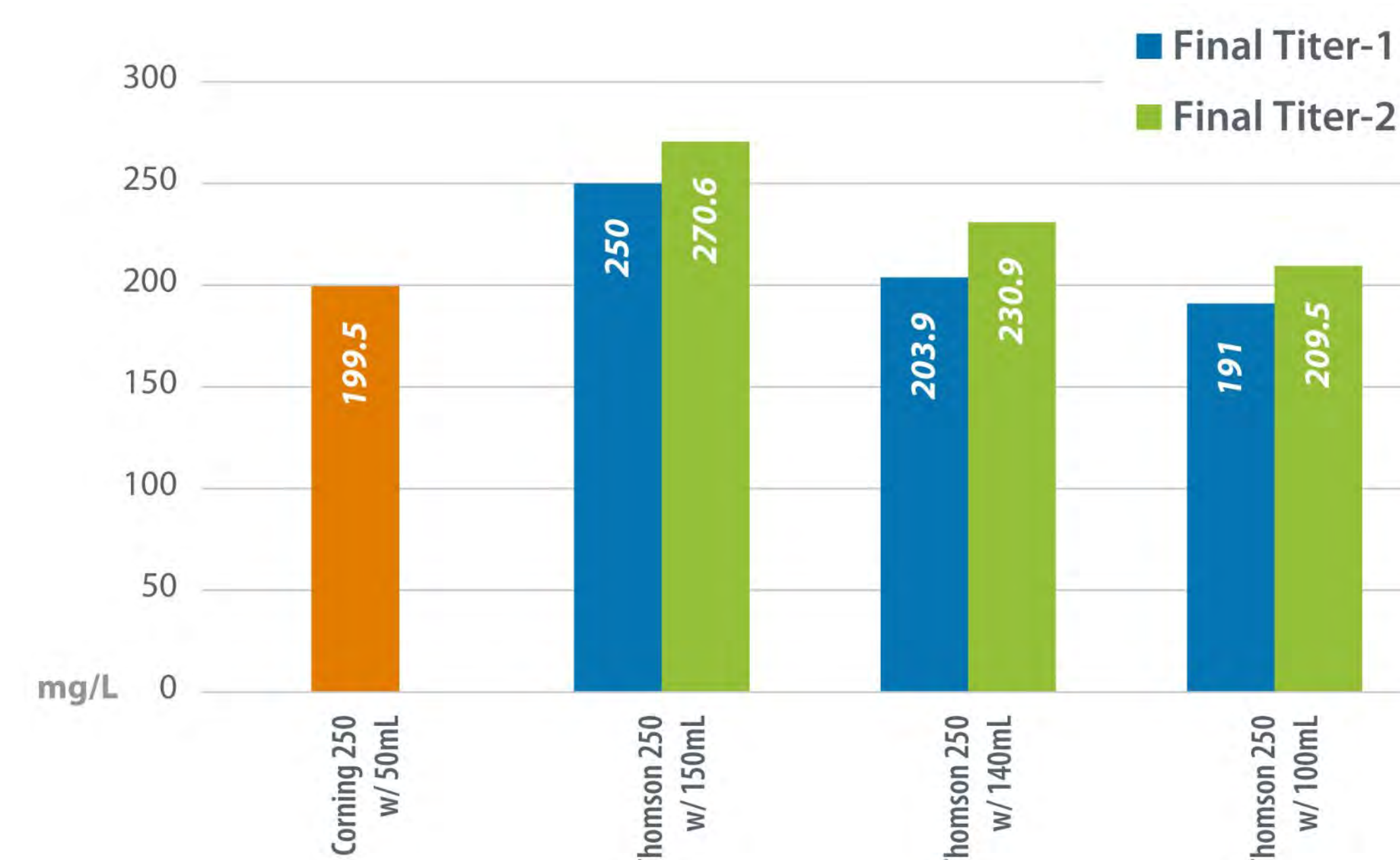


Fig 1. HEK 293 Day 6 Titer - 150rpm

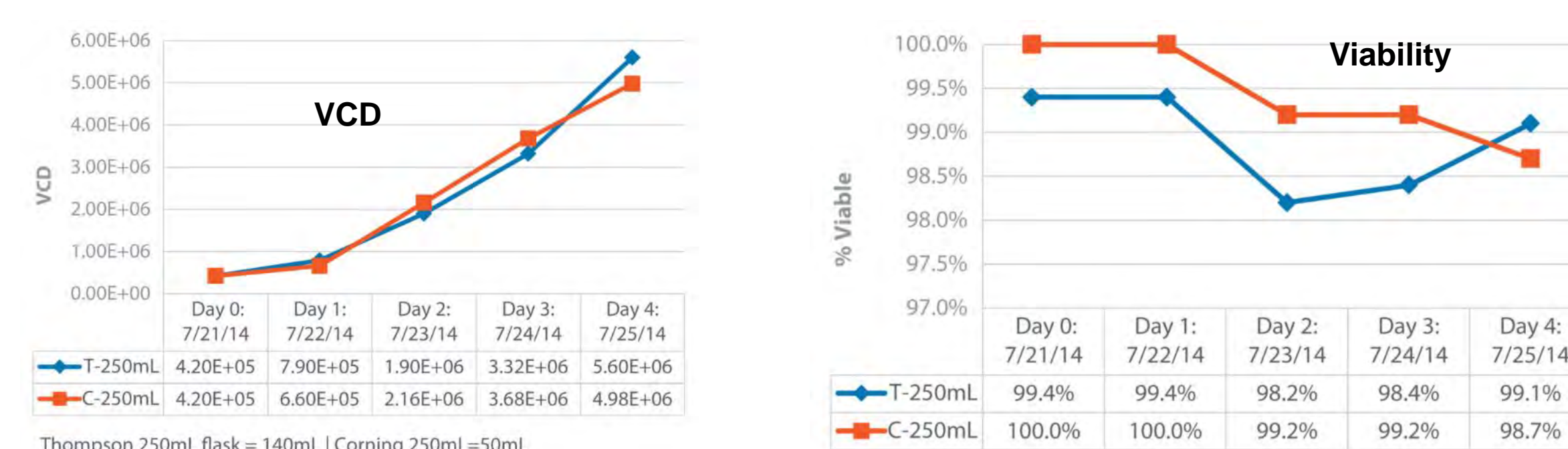


Fig 2. The VCD and cell viability of the Thomson 250mL Optimum **Growth™** Flask with 140mL of working volume were comparable to the Corning® 250mL flask with 50mL of working volume.

Thomson Instrument Company is not affiliated with Agilent, Infors, Axygen, Corning, or their products