Automation of high-throughput titer assays for cell line development

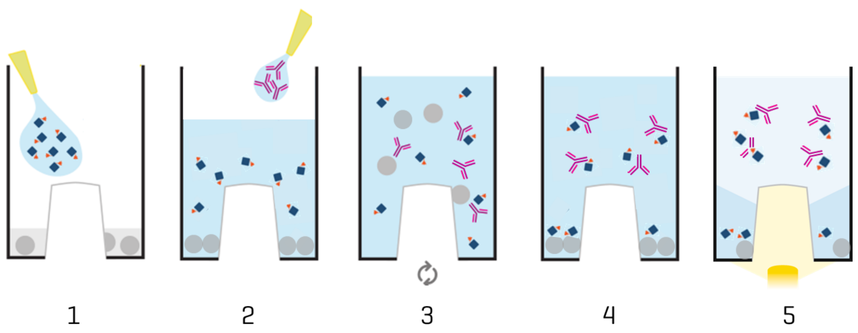
Christian Meissner and Sebastian Giehring

PAIA Biotech GmbH • *Gottfried-Hagen-Straße 60-62 • 51105 Köln • +49 (0) 221 - 168 623 80 • www.paiabio.com*

Abstract

High-throughput methods for screening and automation in bioprocess development have been introduced into most industrial labs over the past years and paved the way for screening larger sample numbers, resulting in higher product yields as well as shorter development time lines and reduced costs. Depending on the specific needs, automation may integrate several parts of the workflow, e.g. clone imaging, hit picking, titering and different liquid handling operations associated with these tasks. Automation solutions can therefore reach from partly or semi-automated to fully automated, which may require huge capital investments.
Here we present how automated PAIA assays compare to Forte Bio Octet measurement and show that rather new and relatively inexpensive liquid handling systems are capable of handling the sample preparation of PAIA assays and with good speed and accuracy. Together with the Cellavista imager (SynenTec GmbH), which serves as the reader, PAIA assays offer an affordable solution for implementing high throughput titer screening with very low investment.

Assay workflow



1 - Addition of 35µL PAIA mix reagent per well
2 - Addition of 5µL cell culture supernatant
3 - Shaking on orbital shaker for 15 mins
4 - Bead settling 5 mins / centrifugation 1 min
5 - Read out on Cellavista/NyONE or plate reader

Figure 1. Workflow steps for the PA-104 Fc low titer assay
This protocol is carried out in the 384 well PAIA*plates*. All steps can be automated if a robotic arm is used to transfer the plates to the orbital shaker and the reader.

Integration with Cellavista imager

	Selection and cloning	Expansion	Media optimization	Bioreactor studies
Single cell assurance	✓	✓		
Confluence	✓	✓		
Trypan blue	✓	✓	✓	✓
Titer PAIA		✓	✓	✓
Glycosylation PAIA			✓	✓

Table 1. The combination of the Cellavista imager with PAIA assays allows to run multiple applications in the cell line development process on one instrument.

Benchmarking studies with established liquid handlers

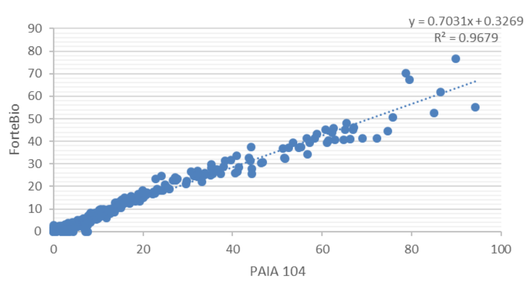


Figure 2. Screening of mini-pools from 96-well plates with the PA-104
Sample preparation was carried out on a Lynx (Dynamic Devices), read-out with Cellavista. Comparison with ForteBio Octet with Protein A tips.

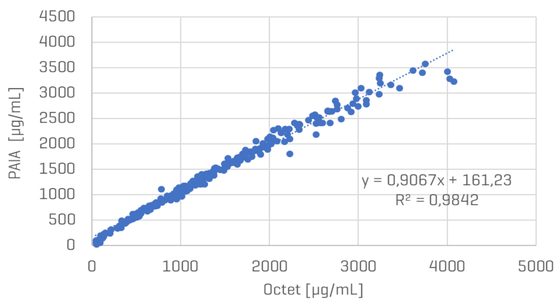
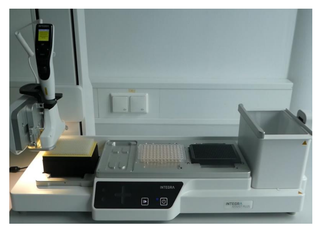


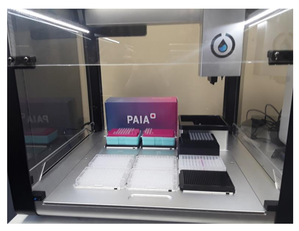
Figure 3. Clone Screening from 96-deep well plates with the PA-104
Sample preparation was carried out on a Microlab Star (Hamilton), read-out with Cellavista. Comparison with ForteBio Octet with Protein A tips.

Low-cost automation solutions

Viaflo Assist Plus



OT-2



Model	Viaflo Assist Plus	OT-2
Manufacturer	Integra Biosciences	Opentrons
Deck positions	4, plus tip rack and waste	11, plus waste
Pipet head options	exchangeable pipets from Viaflo series (flexible tip spacing available)	exchangeable pipets (fixed tip spacing)
Programming	separate software and transfer onto the pipet	in Python
Pipetting accuracy 50µL PAIA*Mix* reagent	CV = 2%	CV = 2%
Pipetting accuracy 5µL Sample	CV = 2%	CV = 1,5 %
Workflow time [preparation of 4 96 well plates]	45 minutes using one 8 and one 16 channel pipets	30 minutes using two 8 channel pipets
No. of manual interventions	4, for exchange of plates and pipets	none

Table 2. Technical features and performance characteristics of low-cost liquid handlers.

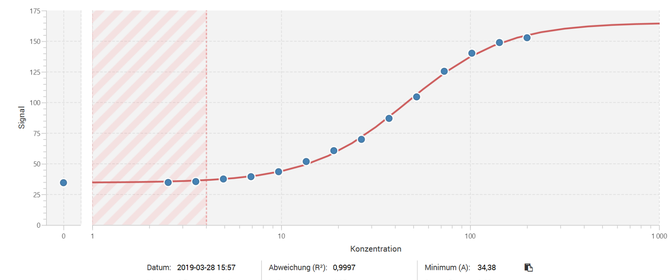


Figure 4. Example of a calibration curve for the PA-104 in cell culture medium
Sample preparation with the OT-2, CV for the triplicates of recalculated samples were all smaller than 5%. Read out was done with a Cellavista, data analysis was performed with PAIA Evaluation Tool.

Discussion

The use of low-cost liquid handlers such as the OT-2 and the Viaflo Assist Plus provide cost efficient automation for the sample preparation for PAIA assays. The OT-2 offers more deck space and is advantageous in a typical workflow where e.g. samples from 96 well formats have to be transferred into a 384 well PAIA*plate*. The OT-2 is not much slower than the Hamilton Star or the Lynx system, unless these are equipped with 96 channel heads, which provides additional time savings.
All solutions presented here allow to perform PAIA assays in less than 100 minutes from start to finish.