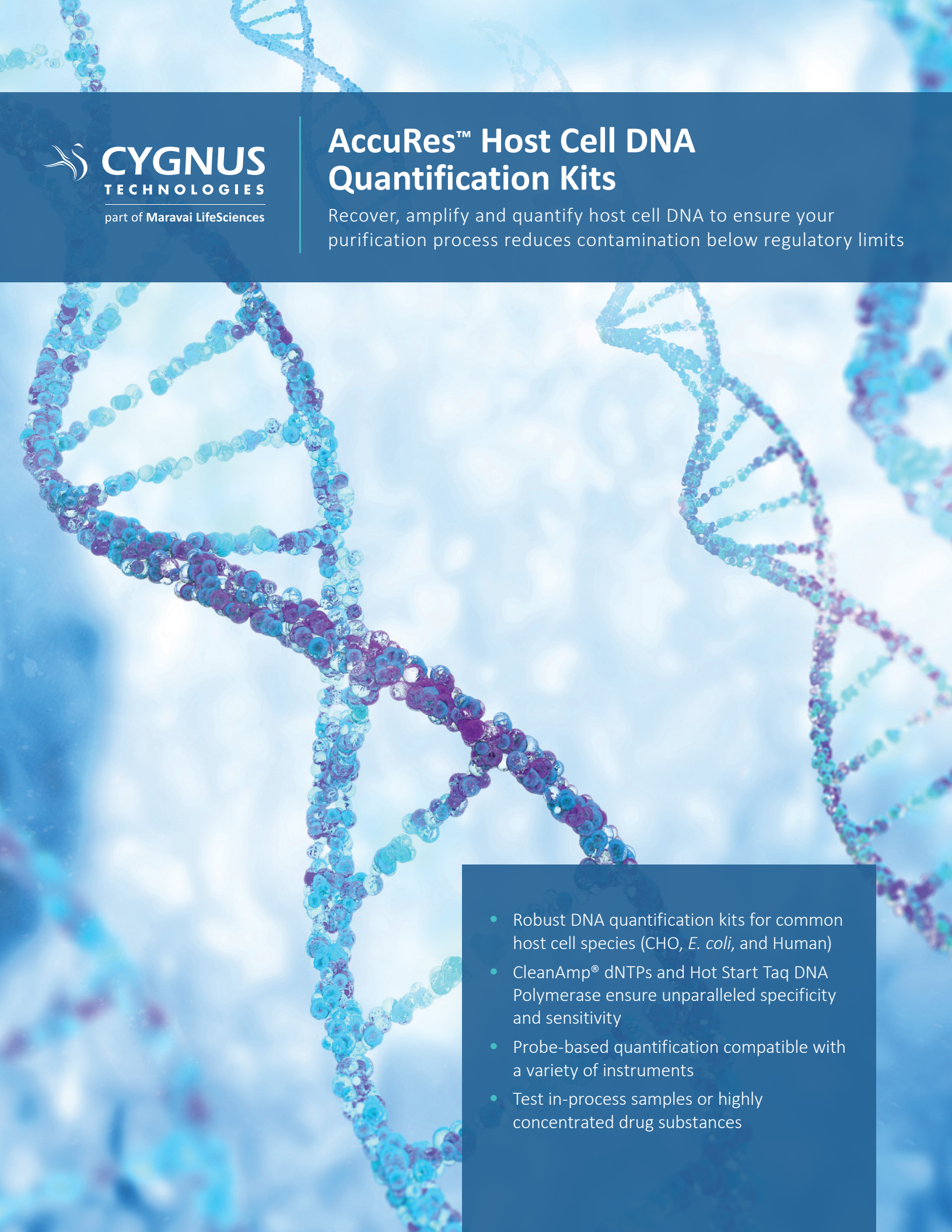
CYGNUS TECHNOLOGIES

part of Maravai LifeSciences

AccuRes™ Host Cell DNA Quantification Kits

Recover, amplify and quantify host cell DNA to ensure your purification process reduces contamination below regulatory limits



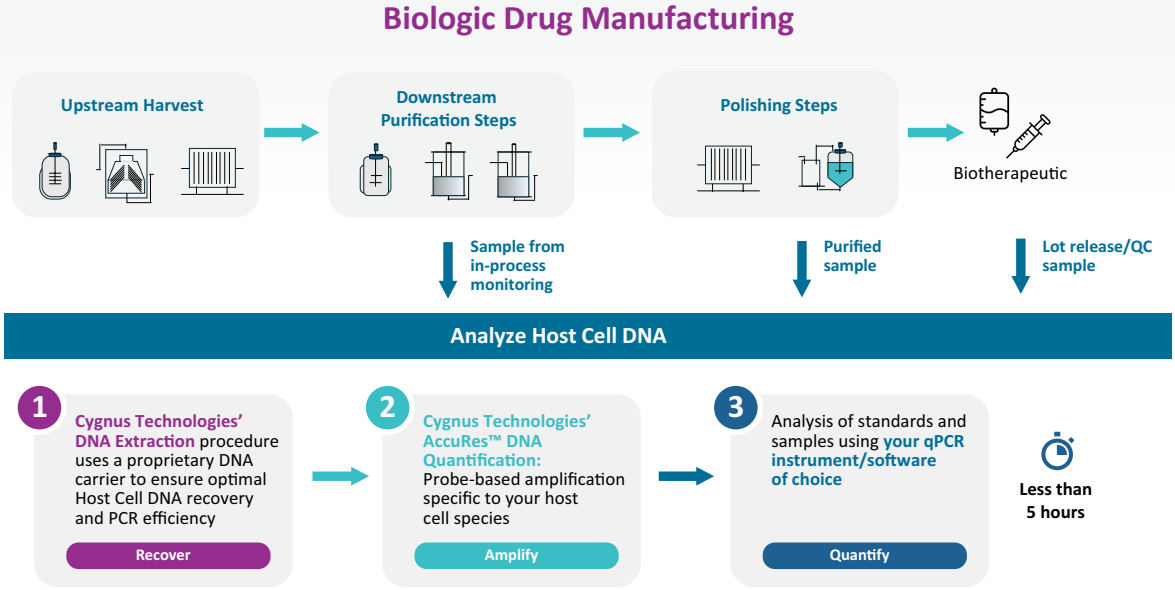
- Robust DNA quantification kits for common host cell species (CHO, *E. coli*, and Human)
- CleanAmp® dNTPs and Hot Start Taq DNA Polymerase ensure unparalleled specificity and sensitivity
- Probe-based quantification compatible with a variety of instruments
- Test in-process samples or highly concentrated drug substances

Detection and Quantification of Residual Host Cell DNA

Host cell DNA can be retained during production of biotherapeutics, which poses a risk for the transfer of oncogenes and/or other problematic genetic material into the final drug substance. To minimize this risk, regulatory agencies have set allowable limits between 10-100 pg/dose, depending on the cell line used and dosing regimen. USP General Chapter <509> "Residual DNA Testing" recommends use of probe-based DNA quantification as a validated method for testing recombinant therapeutic products produced in either *Escherichia coli* (*E. coli*) or Chinese hamster ovary (CHO) cell lines, ensuring better sensitivity and accuracy.

DNA levels can be assessed throughout the manufacturing process; these samples may contain other impurities and a high concentration of drug substance, necessitating a robust and reliable method for accurate and sensitive DNA quantification.

Cygnus Technologies' AccuRes™ DNA Quantification Kits are specially formulated to measure the level of host cell DNA impurities in products manufactured by recombinant expression in CHO, human or *E.coli* cell lines. Samples with very high concentrations of drug substance can be tested with minimal dilution, effectively lowering the LOD of the assay.



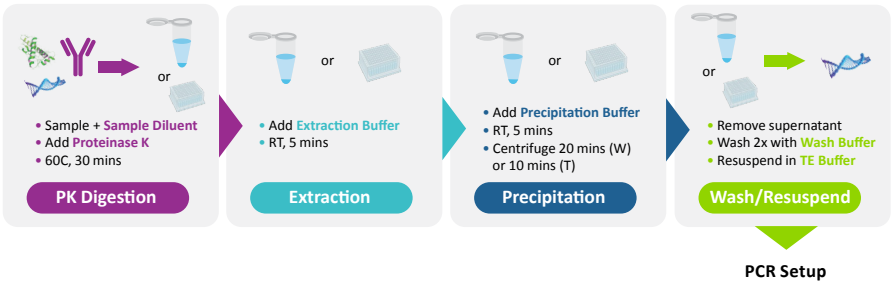
AccuRes™ Quantification Kit Benefits

- **Probe-based DNA quantification method recommended by USP General Chapter <509> "Residual DNA Testing"**
- **Accuracy**—Cygnus' proprietary DNA extraction reagents remove PCR interfering components
- Better **precision**—CleanAmp® dNTPs and Hot Start Taq DNA Polymerase ensure unparallelled specificity and sensitivity
- Demonstrated **sensitivity** with an LOD of 0.6 fg/µL (CHO), 0.7 fg/ µL (*E. coli*), 3 fg/uL (human)
- **Flexibility**—compatible with any qPCR instrument that detects FAM signal, eliminating the cost of purchasing additional equipment or reagents
- **Multiple formats**—CHO AccuRes DNA Quantification Kits take you from sample to PCR and include all reagents for DNA Extraction, AccuRes PCR master mix, primer/probe mix, and DNA standard. CHO, *E. coli*, and Human AccuRes Quantitative DNA Kits include qPCR reagents only, and can be paired with your extraction method of choice.
- **Economical** alternative to established quantitative host cell DNA kits

AccuRes™ DNA Quantification Assay Workflow

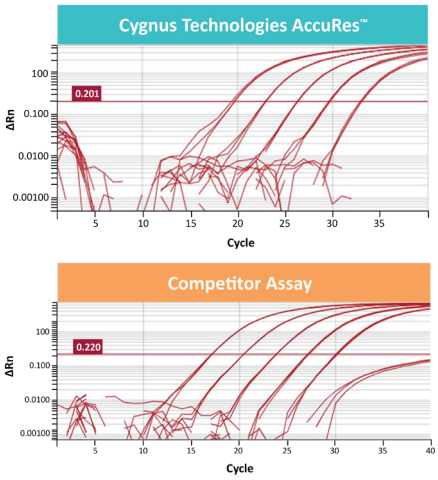
1 Recover

Cygnus Technologies' DNA extraction procedure (available in **tubes [T]** or **wells [W]** format) utilizes a novel DNA carrier to recover **femtogram levels** of residual DNA and perform the measurements in an environment free from contaminating proteins, salts and detergents. This improves reproducibility and robustness of DNA detection and amplification compared to other recovery methods.



2 Amplify

Cygnus Technologies' AccuRes™ kit reagents amplify host cell DNA with a primer/probe mix highly specific for the target host cell species. The FAM-labeled nucleic acid probe is quenched by BHQ-1™ until PCR extension. The **state-of-the-art** master mix includes **CleanAmp® dNTPs** (which includes dUTP) and **Hot Start Taq DNA polymerase** to reduce non-specific amplification, ensuring specificity, sensitivity, and robustness of PCR while allowing the assay to be prepared at ambient temperature.



Standard curves for CHO assays run at specified concentrations. While the efficiency and sensitivity of AccuRes™ reagents is similar to Competitor on control CHO DNA, non-specific amplification of negative controls (arrow) is minimized due to inclusion of CleanAmp® dNTPs and Hot-Start Taq Polymerase.

3 Quantify

The C_T values of the kit standards provided are used to construct a standard curve. The concentration of host cell residual DNA can be mathematically transformed for reporting residual DNA in ng/mL, ng/mg of drug product or in ng/dose. Using this method, residual DNA as low as **1 femtogram per µL** can be measured.

Assay	LOD	LOQ
CHO AccuRes™ Quantitative DNA Kits (Cat. No. D1555/T/W)	0.6 fg/µL	1 fg/µL
***E. coli* AccuRes™ Quantitative DNA Kits (Cat. No. D1415)**	0.7 fg/µL	1 fg/µL
Human AccuRes™ Quantitative DNA Kits (Cat. No. D1165)	3 fg/µL	30 fg/µL

Limit of detection (LOD) and limit of quantification (LOQ) of Cygnus AccuRes™ kits.

CHO Assay Performance

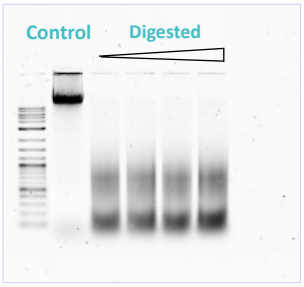
Intra-Assay Precision

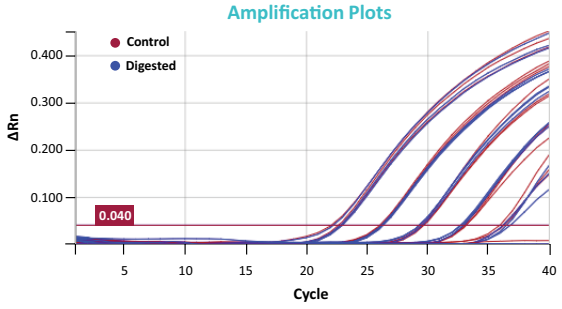
DNA Conc.	Mean Conc. A	SD A	CV% A	Mean Conc. B	SD B	CV% B	Mean Recovery
0.05 pg	0.04	0.005	12%	0.06	0.004	5.7%	**105%**
0.5 pg	0.39	0.006	1.5%	0.54	0.013	2.4%	**93%**
5 pg	4.20	0.309	7.3%	4.69	0.150	3.2%	**89%**
50 pg	46.45	1.456	3.1%	48.67	1.988	4.1%	**95%**

Inter-Assay Precision

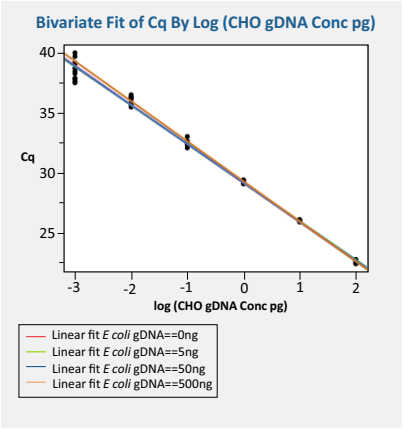
DNA Conc.	Mean Conc.	SD	CV%
0.05 pg	0.05	0.003	5.5%
0.5 pg	0.47	0.007	1.5%
5 pg	4.44	0.116	2.6%
50 pg	47.56	1.618	3.4%

Intra- and Inter-Assay Precision.
Known CHO DNA samples were assayed in duplicate by 2 operators (A and B) over 2 days. SD, CV, and % recovery were calculated.

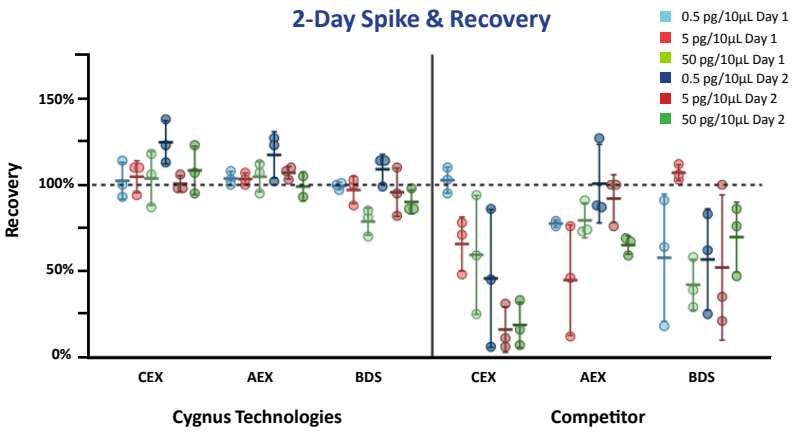




Recovery and amplification of intact and fragmented CHO DNA. Gel image of intact control gDNA and digested gDNA treated with Alu1 for 30 min, 1 hour, 1.5 hours, and 2 hours. Amplification of control and digested (30 min) samples is similar across the linear range.



Broad linear range and specificity of CHO primer/probe in the presence of foreign DNA.
Presence of *E. coli* DNA at varying concentrations does not impact the CHO DNA standard curve.



Superior spike and recovery of CHO DNA extracted and amplified with Cygnus Technologies AccuRes™ Quantification kit (D1555W) versus Competitor manual extraction and qPCR protocol. Highly robust and reproducible recovery of in-process samples (CEX, AEX) and drug substances (BDS) was observed with the Cygnus extraction and quantification kit as opposed to the Competitor kit.

Human Assay Performance

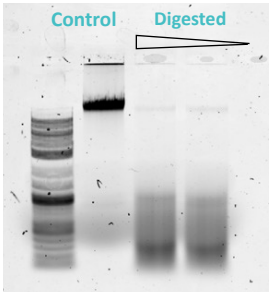
Intra-Assay Precision

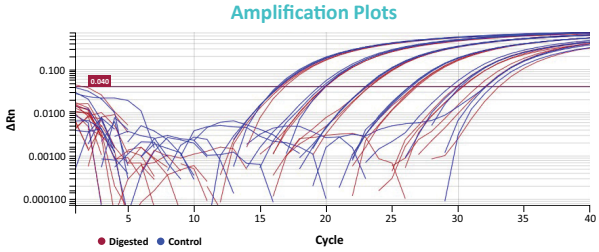
DNA Conc.	Mean Conc. A	SD A	CV% A	Mean Conc. B	SD B	CV% B	Mean Recovery
1.5 pg	1.221	0.353	2.9%	1.361	0.059	4.3%	**86%**
15 pg	14.98	0.287	1.9%	15.72	0.613	3.9%	**102%**
150 pg	155.7	4.981	3.2%	168.3	6.441	3.8%	**108%**
1500 pg	1521	208.6	13.7%	1398	84.47	6.0%	**97%**

Inter-Assay Precision

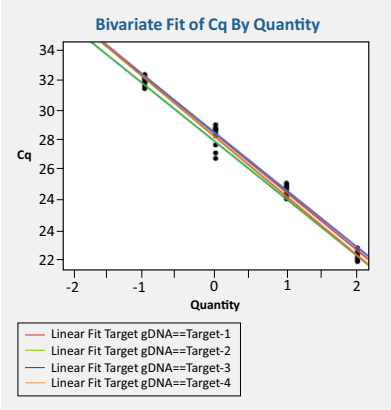
DNA Conc.	Mean Conc.	SD	CV%
1.5pg	1.29	0.04	2.9%
15pg	15.35	0.29	1.9%
150pg	161.97	4.86	3.0%
1500pg	1459.2	113.21	7.8%

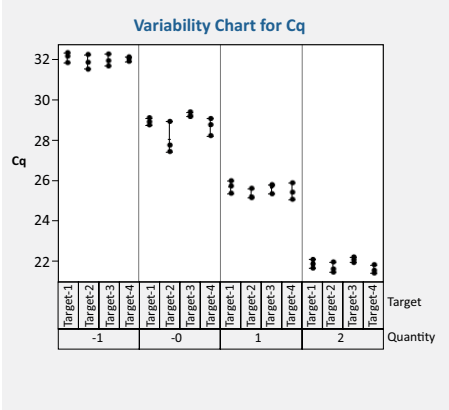
Intra- and Inter-Assay Precision.
Four human DNA samples were assayed in triplicate by 2 operators (A and B) over 2 days. SD, CV, and % recovery were calculated.





Recovery and amplification of intact and fragmented CHO DNA. Gel image of intact control gDNA and digested gDNA treated with Alu1 for 15 min or 30 min. Amplification of control and digested (30 min) samples is similar across the linear range.





Target		Foreign DNA Concentration
1	Human gDNA only	
2	CHO gDNA	50ng
3	CHO gDNA	5ng
4	CHO gDNA	0.5ng

Broad linear range and specificity of human AccuRes™ primer/probe in the presence of foreign DNA.
Presence of CHO DNA at varying concentrations does not impact the human DNA standard curve.

E.coli Assay Performance

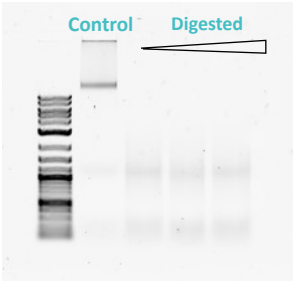
Intra-Assay Precision

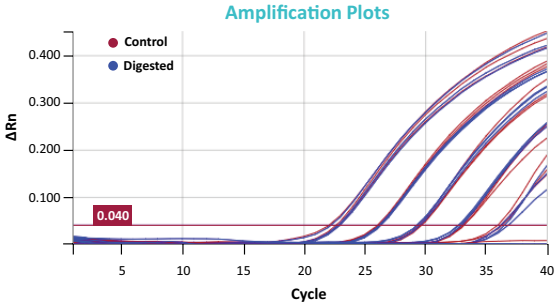
DNA Conc.	Mean Conc. A	SD A	CV% A	Mean Conc. B	SD B	CV% B	Mean Recovery
0.05 pg	0.045	1.142	4.3%	0.480	0.004	9.4%	**89%**
0.5 pg	0.481	0.196	2.4%	0.440	0.021	4.8%	**89%**
5 pg	4.540	0.011	4.3%	4.510	0.251	5.7%	**94%**
50 pg	47.14	0.002	2.4%	40.29	7.768	19%	**89%**

Inter-Assay Precision

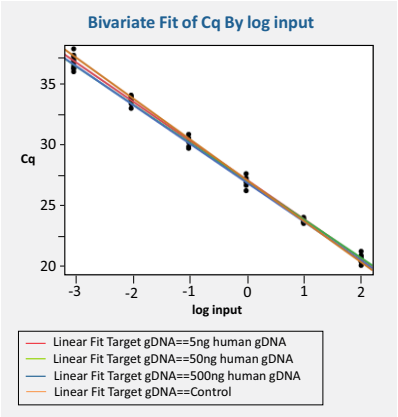
DNA Conc.	Mean Conc. A	SD	CV%
0.05 pg	0.05	0.00	5.1%
0.5 pg	0.46	0.02	3.6%
5 pg	4.52	0.18	3.9%
50 pg	43.72	4.44	10.2%

Intra- and Inter-Assay Precision.
Three *E.coli* DNA samples were assayed in triplicate by 2 operators (A and B) over 2 days. SD, CV, and % recovery were calculated.





Recovery and amplification of intact and fragmented *E.coli* DNA. Gel image of intact control gDNA and digested gDNA treated with Alu1 for 15 min, 30 min, 1 hour. Amplification of control and digested (30 min) samples is similar across the linear range.



Broad linear range and specificity of *E.coli* primer/probe in the presence of foreign DNA. Presence of CHO DNA at varying concentrations does not impact the *E. coli* DNA standard curve

Sample	DNA Input	DNA Measured	% Recovery
Anion Ex. (1 mg/mL protein)	50 pg/10 µL 5 pg/10 µL 0.5 pg/10 µL	37 pg/10 µL 5.24 pg/10 µL 0.51 pg/10 µL	74% 105% 101%
Cation Ex. (1 mg/mL protein)	50 pg/10 µL 5 pg/10 µL 0.5 pg/10 µL	54 pg/10 µL 5.19 pg/10 µL 0.49 pg/10 µL	108% 104% 98%
DS (1 mg/mL protein)	50 pg/10 µL 5 pg/10 µL 0.5 pg/10 µL	59.7 pg/10 µL 5.88 pg/10 µL 0.61 pg/10 µL	119% 118% 122%

Assay performance using in-process and Drug Substance (DS) samples.
Spike recovery of *E.coli* DNA containing a 1 mg/mL AEX or CEX pooled sample or DS matrix.

Ordering Information

AccuRes™ Kit Information

Product	Cat. No.
CHO AccuRes™ DNA Quantification Kit in Tubes	D1555T
CHO AccuRes™ DNA Quantification Kit in Wells	D1555W
CHO AccuRes™ Quantitative DNA Kit	D1555
Human AccuRes™ Quantitative DNA Kit	D1165
E. coli AccuRes™ Quantitative DNA Kit	D1415
Vero AccuRes™ Quantitative DNA Kit	D1975
NS/0 AccuRes™ Quantitative DNA Kit	D1225
SF9 AccuRes™ Quantitative DNA Kit	D1845
P. pastoris AccuRes™ Quantitative DNA Kit	D1145

AccuRes™ Kit Components

DNA Extraction (D1555T/W Kits only)	DNA Amplification (All kits)
Proteinase K	DNA Concentrate
DNA Extraction Buffer	AccuRes™ PCR Master Mix, 2X
DNA Precipitation Buffer	AccuRes™ Primers and Probe Mix, 10X
DNA Wash Buffer	AccuRes™ Deionized Water
DNA Sample Diluent	PCR Assay Plate
Tubes or 96 well plate	DNA TE Buffer (Quantitative Kits only)
2mL Sterile Microfuge Tubes	

Related Products for DNA Extraction

DNA Extraction kits can be purchased separately for use with our AccuRes™ Quantitative kits.

Product	Cat. No.
DNA Extraction Kit in Tubes	D100T
DNA Extraction Kit in Wells	D100W

Support and Services

Cygnus Technologies' experts offer:

- Sample evaluation services and technical support for your DNA assays.
- Development of residual host cell DNA kits specific to your expression system.

Scan to view our AccuRes™ products and place an order.

Contact our team for more information or to develop a custom assay.

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Cygnus Industry-leading HCP ELISA and Other Process Impurity Kits

HCP ELISA Kits & Automated Solutions

Product	Cat. No.
CHO HCP ELISA Kit, 3G	F550-1
CHO Lysate Host Cell Protein ELISA Kit, 2G	F1045
HEK 293 HCP ELISA Kit, 3G	F650S
Sf9 HCP ELISA Kit, 3G	F1040
NS/0 HCP ELISA Kit	F220
E. coli HCP ELISA Kit, 2G	F1020
BL21(DE3) HCP ELISA Kit	F1060
E. coli HCP ELISA Kit	F410
Vero Cell 2G HCP ELISA Kit	F975

Product	Cat. No.
CHO HCP ELISA 3G, Robotics Kit	F550-1-4
CHO HCP Simple Plex Assay, 3G-1	SSPCKB-OT-003714
CHO HCP 3G Assay Reagent Set for Gyrolab®	G550-1
CHO HCP 3G Assay Reagent Set for Gyrolab®, CD5	G550-1-5
HEK 293 HCP ELISA 3G, Robotics Kit	F650S-4
Simple Plex HEK 293 HCP 3G Assay	SPCKB-OT-007066
HEK 293 HCP Assay Reagent Set for Gyrolab®	G650S-1
E. coli HCP 2G Assay Reagent Set for Gyrolab®	G1020
Sf9 HCP 3G Assay Reagent Set for Gyrolab®	G1040

Bioprocess Impurity ELISA Kits

Product	Cat. No.
EndonucleaseGTP® ELISA	F960
EndonucleaseGTP® Assay Reagent Set for Gyrolab®	G960
Protein A Mix-N-Go ELISA	F600
Protein A Mix-N-Go ELISA, for UnNatural Protein A Constructs	F610
Protein A Mix-N-Go ELISA, for Amsphere™ ligands	F740
Tosoh R40 and R28 Protein A Mix-N-Go ELISA	F910
KANEKA KanCapA™ 3G Protein A Mix-N-Go ELISA	F950
J.T.Baker® BakerBond® PROchievA™ Protein A Mix-N-Go ELISA	F965

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