

Residual Host Cell DNA Analysis Tools



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Residual host cell DNA contamination can be introduced during production of biotherapeutics. Due to the theoretical potential for the transfer of oncogenes from the host cells, regulatory agencies have set allowable limits between 100 pg/dose and 10 ng/dose depending on the cell line used as well as the mode and frequency of dosing. These requirements demand a sensitive method of DNA detection for compliance. Building on its expertise in host cell protein analysis, Cygnus Technologies developed a broad range of residual host cell DNA analysis tools.

Host Cell DNA Extraction Kits

Cygnus Technologies' DNA Extraction Kits are based on a proprietary DNA extraction procedure to recover <1pg/mL residual DNA and perform the measurements in an environment free from contaminating proteins, salts and detergents. As a result, reproducibility and robustness of DNA detection and amplification assays are improved.

- Contain novel DNA carrier designed for host cell DNA recovery from drug substance samples
- Allow precise 80-120% recovery of low-concentration residual host cell DNA
- Easy DNA pellet handling for reproducible results—does not require a highly-skilled technician to perform extractions
- Kits are available in tube and 96-well formats, and include all reagents necessary to perform up to 100 extractions
- Compatible with downstream DNA detection and amplification assays

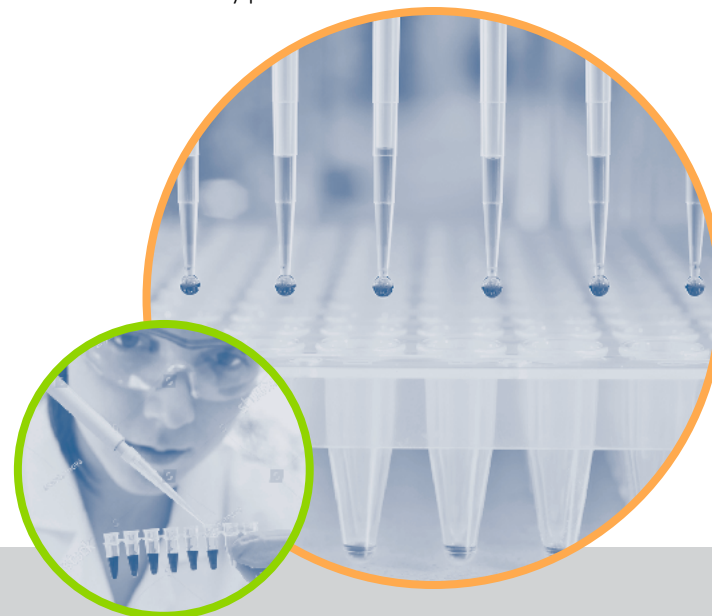
Ordering Information

Product	Catalog No.	Price (\$)
DNA Extraction Kit in Tubes	D100T	288.75
DNA Extraction Kit in Wells	D100W	288.75
CHO DNA Amplification Kit in Tubes	D555T	761.25
CHO DNA Amplification Kit in Wells	D555W	761.25
<i>E. coli</i> DNA Amplification Kit in Tubes	D415T	729.75
<i>E. coli</i> DNA Amplification Kit in Wells	D415W	729.75
CHO Host Cell DNA Detection Kit in Tubes	D550T	761.26
CHO Host Cell DNA Detection Kit in Wells	D550W	761.25
<i>E. coli</i> Host Cell DNA Detection Kit in Tubes	D410T	656.25
<i>E. coli</i> Host Cell DNA Detection Kit in Wells	D410W	656.25
Human Host Cell DNA Detection Kit in Tubes	D160T	656.25
Human Host Cell DNA Detection Kit in Wells	D160W	656.25
NS/O Host Cell DNA Detection Kit in Tubes	D220T	656.25
NS/O Host Cell DNA Detection Kit in Wells	D220W	656.25

Host Cell DNA Detection Kits

Cygnus Technologies' DNA Detection Kits are designed to measure residual host cell DNA during process development and for in-process monitoring. These DNA dye binding assays utilize PicoGreen® dye, a DNA intercalator that binds strongly to double stranded DNA. While PicoGreen®-based assays have been employed by biopharmaceutical manufacturers for years, in many cases proteins and buffer components could interfere in PicoGreen® dye binding to DNA, resulting in either over- or under- estimation of the true DNA concentration. Based on Cygnus' proprietary DNA extraction procedure to isolate the residual DNA, the PicoGreen®-based detection is performed in an environment free from contaminating proteins, salts and detergents. The kits' detection range is from 400 pg/mL to 200 ng/mL (CV <15%. LLOQ is determined at 500 pg/mL). Cygnus Technologies offers kits for detection of residual CHO, human, *E. coli* and NS/O host cell DNA.

- Reliable tool for process development and in-process monitoring
- Based on Cygnus' proprietary residual host cell DNA extraction method
- Include cell-specific DNA Standard Sets
- Available in tube or 96-well format for DNA extraction step
- Kits include the assay plate

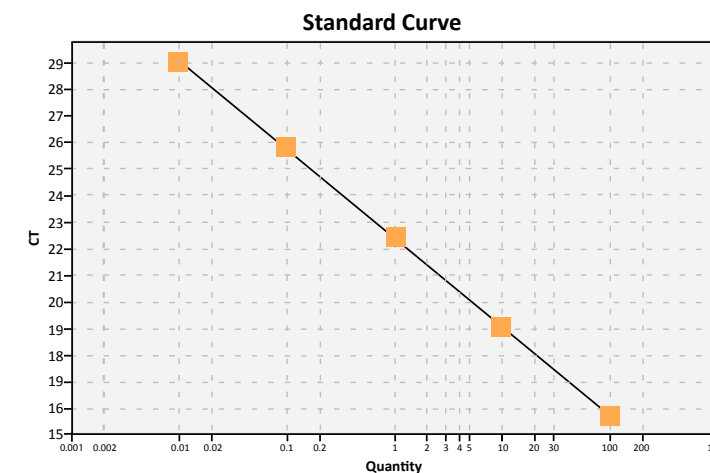


Host Cell DNA Amplification Kits

Cygnus Technologies' Host Cell DNA Amplification Kits are used to measure the level of host cell DNA contamination in products manufactured by recombinant expression in CHO or *E. coli* cell lines. Samples with very high concentrations of drug substance can be tested with minimal dilution, effectively lowering the LLOQ of the assay when compared to similar methods on the market.

These convenient, easy-to-use kits are compatible with customers' existing qPCR reagents* and instruments. Each kit includes all materials required for Cygnus' proprietary extraction of DNA to remove PCR interfering components, DNA concentrate for standards, a qualified cell-line specific primer set, and a PCR assay plate with optical seal. Residual DNA can be measured to 1 part per billion when using this kit.

- Based on Cygnus' proprietary residual host cell DNA extraction method
- Include cell-specific DNA standard and primer sets
- Available in tube or 96-well format for DNA extraction step
- Compatible with existing qPCR reagents and instruments



*Cygnus recommends Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Manufacturer Cat# 4368706, 2 x 5 mL.) or a master mix from another licensed vendor.

Table 1: DNA Spike Recovery — Cygnus

Sample	Dilution	Result	Average Result	Spike	Average Spike	Std Dev	%CV	Spike Recovery	Dilution Corrected Result (pg/10 µL)	Average Result (pg/10 µL)	Reported Result (ng/mL)	Reported Result (ng/mg)
Ion Exchange (IgG, ~5mg/mL)	5	0.161	0.1600	24.3	24.05	0.354	1.5%	96%	0.8	0.817	0.082	0.0163
		0.159		23.8								
	10	0.08	0.0810	10.93	11.08	0.212	1.9%	88%	0.81			
		0.082		11.23								
	20	0.041	0.0420	5.63	5.65	0.028	0.5%	90%	0.84			
		0.043		5.67								
Protein A Pool (IgG, ~8mg/mL)	5	19.38	19.6400	47.29	47.34	0.071	0.1%	111%	98.2	103.417	10.342	1.2927
		19.9		47.39								
	10	10.57	10.5250	21.19	21.21	0.028	0.1%	85%	105.25			
		10.48		21.23								
	20	5.3	5.3400	11.17	11.275	0.148	1.3%	95%	106.8			
		5.38		11.38								
Drug Substance Y (IgG, ~50mg/mL)	5	3.1	3.1000	28.17	27.865	0.431	1.5%	99%	15.5	17.917	1.792	0.0358
		3.1		27.56								
	10	1.86	1.8100	12.91	13.035	0.177	1.4%	90%	18.1			
		1.76		13.16								
	20	1.03	1.0075	6.61	6.67	0.085	1.3%	91%	20.15			
		0.985		6.73								

Table 2: DNA Spike Recovery — Competitor W

Sample	Dilution	Result	Average Result	Spike	Average Spike	Std Dev	%CV	Spike Recovery	Dilution Corrected Result (pg/10 µL)	Average Result (pg/10 µL)	Reported Result (ng/mL)	Reported Result (ng/mg)
Ion Exchange (IgG, ~5mg/mL)	5	0.109	0.1085	7.61	7.705	0.134	1.7%	30%	Bad Spike	0.762	0.0762	0.010
		0.108		7.8								
	10	0.0367	0.0380	5.55	5.68	0.184	3.2%	45%	Bad Spike			
		0.0392		5.81								
	20	0.0382	0.0381	3.87	3.915	0.064	1.6%	62%	0.762			
		0.038		3.96								
Protein A Pool (IgG, ~8mg/mL)	5	22.24	22.3050	46.04	45.71	0.467	1.0%	94%	111.525	113.86	11.386	1.423
		22.37		45.38								
	10	10.24	10.2100	11.05	10.825	0.318	2.9%	5%	Bad Spike			
		10.18		10.6								
	20	5.69	5.8100	10.45	10.285	0.233	2.3%	72%	116.2			
		5.93		10.12								
Drug Substance Y (IgG, ~50mg/mL)	5	3.39	3.4500	23.01	22.37	0.905	4.0%	76%	17.25	18.43	1.843	0.0369
		3.51		21.73								
	10	1.76	1.7550	10.72	10.57	0.212	2.0%	71%	17.55			
		1.75		10.42								
	20	1.01	1.0250	5.52	5.505	0.021	0.4%	72%	20.5			
		1.04		5.49								

Table 3: DNA Spike Recovery — Competitor E

Sample	Dilution	Result	Average Result	Spike	Average Spike	Std Dev	%CV	Spike Recovery	Dilution Corrected Result (pg/10 µL)	Average Result (pg/10 µL)	Reported Result (ng/mL)	Reported Result (ng/mg)
Ion Exchange (IgG, ~5mg/mL)	5	Undetermined	NA	31.45	31.32	0.184	0.6%	NA	NA	1.06	0.106	0.0132
		<LLOQ		31.19								
	10	0.1154	0.1181	8.37	8.14	0.325	4.0%	64%	1.1805			
		0.1207		7.91								
	20	0.0461	0.0466	4	4.17	0.240	5.8%	66%	0.932			
		0.0471		4.34								
Protein A Pool (IgG, ~8mg/mL)	5	18.67	19.3300	40.36	39	1.923	4.9%	79%	96.65	101.13	10.113	1.2641
		19.99		37.64								
	10	8.16	8.1050	12.27	12.02	0.354	2.9%	31%	Bad Spike			
		8.05		11.77								
	20	5.26	5.2800	8.6	8.54	0.085	1.0%	52%	105.6			
		5.3		8.48								
Drug Substance (IgG, ~50mg/mL)	5	2.27	2.3800	13.46	13.215	0.346	2.6%	43%	Bad Spike	NA	NA	Poor Spike Recovery at all dilutions
		2.49		12.97								
	10	1.23	1.1750	3.819	3.4695	0.494	14.2%	18%	Bad Spike			
		1.12		3.12								
	20	0.8307	0.8106	3.15	3.1	0.071	2.3%	37%	Bad Spike			
		0.7905		3.05								

Cygnus DNA Extraction Kit in Tubes (Cat# D100T), the Competitor W DNA Extractor Kit and Competitor E Complete DNA and RNA Purification Kit were evaluated with respect to performance and ease of use.

Testing was performed on in-process samples spanning the range of purification processes as well as samples manufactured to simulate clean human IgG Drug Substances. qPCR was performed on extracted samples from all 3 kits using the Cygnus CHO DNA Amplification Kit, Cat# D555T.