Process Sciences

Date: March 5, 2002

TITLE **ARM Assay Report: Assessment of Residual Host Cell Protein**

AUTHOR(S) **Barry Sugarman**

KEY WORDS **ARM**
Wild-type Ad5
Residual HEK 293 Host Cell Protein

REFERENCE **Cygnus Technologies product insert number F150**
Cygnus Technologies product insert number F155

SUMMARY Canji tested the ARMWG wtAd5 sample (ATCC VR-1516, Part Number 10-00023, Lot 001503) for residual 293 Host Cell Protein(s) [HCPs] using two commercially available kits from Cygnus Technologies (Plainville, MA). One was a semi-quantitative 293 HCP ELISA (catalog number F150), whereas the other was a qualitative Western blot method (catalog number F155). In the ELISA assay, the value that was reported was 18.4 ng/mL. In the Western blot assay, bands were observed in sample lanes; however, they did not correspond to 293 HCPs present in the assay control (i.e., uninfected 293 cell lysate).

Reported by: _____ Date: _____
Barry J. Sugarman
Associate Director, Process Sciences

Reviewed by: _____ Date: _____
Beth M. Hutchins
Director, Process Sciences

☐ **Restricted Release**

Residual 293 HCP by ELISA

Procedure: Immunoenzymatic Assay for the Measurement of HEK 293 Host Cell Proteins

Analyst: Josie Beltran

Reviewer: Doug Antelman

Description: One 0.5 mL vial of the purified wtAd5 sample (ATCC VR-1516, Part Number 10-00023, Lot 001503) was used for this test. It was thawed and used for testing on December 17, 2001. The sample was diluted and manipulated as described in the product insert (F150, revision #10-01). The response was assessed at 450 nm using a microplate spectrophotometer (Molecular Devices, Mountain View, CA). Values for 293 HCP were interpolated from the standard curve using SoftMAX Pro software (Molecular Devices, Mountain View, CA). Only sample dilutions whose OD_{450nm} values were greater than 0.1 were included in the calculation of the final result.

Results and Comments:

Five different concentrations of the HEK 293 HCP standard were used to generate a standard curve. These concentrations were 250, 75, 20, 4, and 1 ng/mL; an additional "standard" containing none of the 293 HEK control supplied with the kit was also included. These data were fit using a 4-parameter logistic equation (Figure 1). Each concentration of standard was tested in duplicate wells.

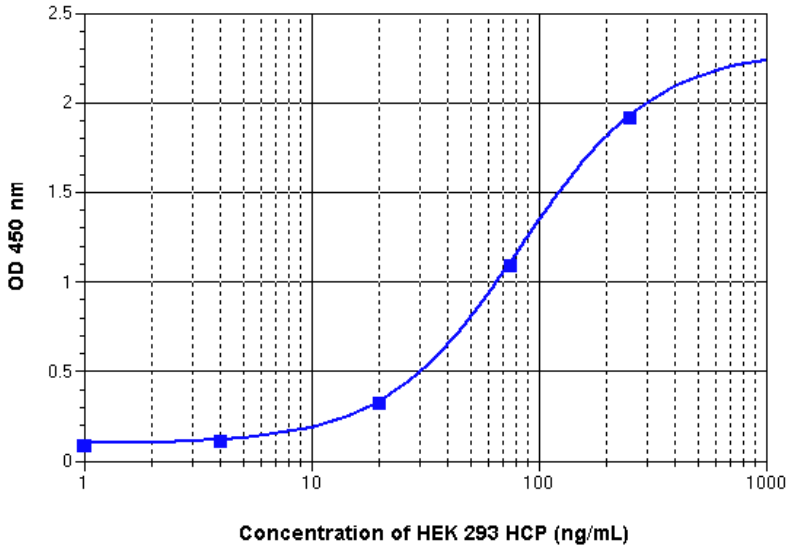

Figure 1. Standard curve generated using the HEK 293 HCP control provided by Cygnus Technologies. The OD_{450nm} response (average value) is depicted as a function of concentration. The ELISA standard was tested at concentrations from 1 to 250 ng/mL.

The ARMWG sample was diluted in 2-fold increments and tested for the presence of HEK 293 HCPs. Each dilution was tested in quadruplicate. The results are listed in Table I. Only OD_{450nm} values greater than or equal to 0.100 were used in the final calculation for the concentration of residual HEK 293 protein(s).

Table I. Data Summary for the HEK 293 HCP ELISA

Dilution Factor	Well ID	OD_{450nm} Value	Result (ng/mL)	Adjusted Result (ng/mL)
2	A3	0.106	4.883	9.766
	A4	0.146	7.973	15.946
	A5	0.131	6.844	13.688
	A6	0.099	< Std	< 8
4	B3	0.066	< Std	< 16
	B4	0.110	5.159	20.636
	B5	0.116	5.697	22.788
	B6	0.132	6.950	27.800

The average of the six values was 18.4±6.6 ng/mL. Although the data suggest that the "relative" concentration of residual HCP was quite low (i.e., < 20 ng/mL), the data suggest that there may have been an inhibitory effect at the highest [particle] concentration tested.

Residual 293 HCP by Western Blot

Procedure: Western Blot Kit for the Detection of HEK 293 Host Cell Proteins

Analyst: Josie Beltran

Reviewer: Doug Antelman

Description: The remainder of the 0.5 mL vial of purified wtAd5 sample (ATCC VR-1516, Part Number 10-00023, Lot 001503) used for the 293 HCP ELISA was used for this analysis. It was thawed and used for testing on February 11 and 12, 2002. The sample was diluted and manipulated as described in the product insert. The sample was denatured and the proteins resolved on a 4-20% Tris-Glycine gradient acrylamide gel and subsequently electrotransferred to nitrocellulose. The blot was blocked overnight and then probed as prescribed within the product insert.

Results and Comments:

Three different amounts of the sample were loaded onto the gel as well as biotinylated molecular weight marker, Rainbow™ molecular weight markers, and two different amounts of the HEK 293 host cell control antigen. The resulting Western blot is depicted in Figure 2.

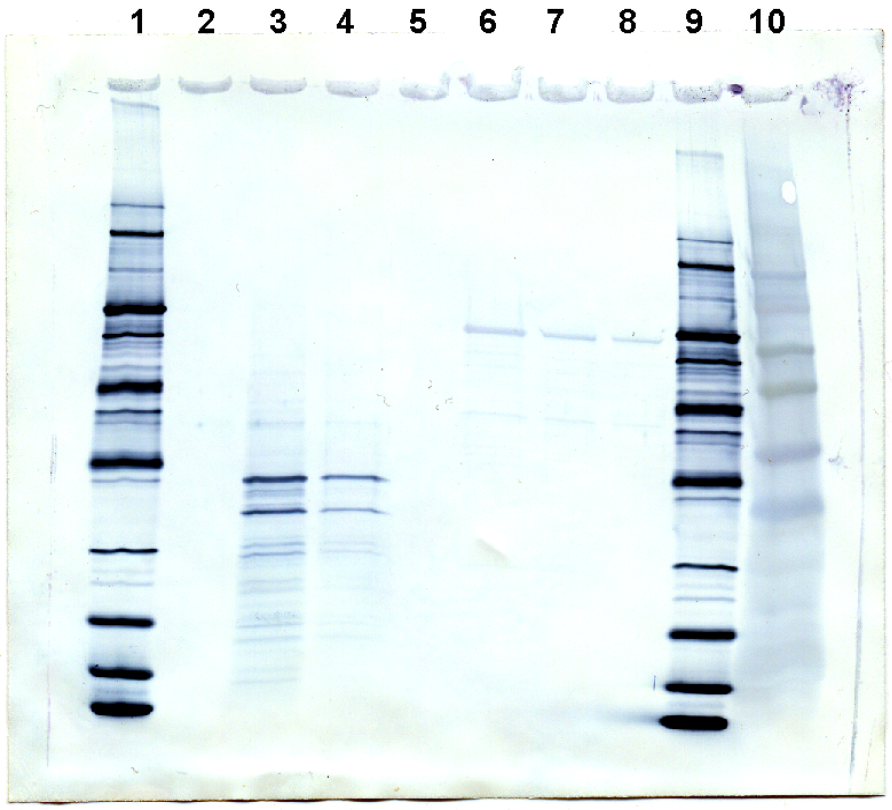

Figure 2. Western blot for the Determination of HEK 293 Host Cell Proteins in the ARMWG wtAd5 sample (ATCC VR-1516, Part Number 10-00023, Lot 001503). The proteins in each marker, assay control or sample were resolved by SDS-PAGE on a 4-20% Tris-Glycine gel, and subsequently electrotransferred to nitrocellulose. The contents of each lane were as follows: lanes 1 and 9, Biotinylated SDS-PAGE Molecular Weight Markers (Bio-Rad, Broad Range, 6.5 to 200 kD); lane 2, ARMWG Excipient Buffer; lanes 3 and 4, HEK 293 host cell antigen diluted either 2- or 5-fold, respectively; lane 5, Empty; lanes 6, 7, and 8, the ARMWG wtAd5 sample diluted either 2-, 4-, or 10-fold, respectively; and lane 10, the Rainbow™ molecular weight markers. The markers in lanes 1 and 9 were as follows: Myosin (200 kD), β-galactosidase (116.25 kD), Phosphorylase b (97.4 kD), Bovine serum albumin (66.2 kD), Ovalbumin (45 kD), Carbonic anhydrase (31 kD), Soybean trypsin inhibitor (21.5 kD), Lysozyme (14.4 kD), and Aprotinin (6.5 kD).

Although a few prominent bands were present in sample lanes, they did not correspond to any bands in the lane containing the 293 HCP assay control (i.e., between 12 and 85 kD). The molecular weight of the bands in lanes 6, 7 and 8 were close to those predicted for two Ad capsid proteins: hexon and penton (i.e., 112 and 98 kD) as well as other Ad derived proteins (92 and 66 kD). It would not be clear as to why the antisera may have bound to these Ad capsid proteins. However, it is plausible that some non-specific interaction (i.e., binding to hydrophobic residues) might explain why this result was observed.

Appendices

- Cygnus Technologies product insert number F150
- Cygnus Technologies product insert number F155