

## Easy, Accurate, Fast Quantification of IgG Antibodies

The process of generating monoclonal antibodies from hybrid cell lines has undergone major advances in recent years. However, the methods available for screening specific antibodies secreted by hybridomas, such as Enzyme-Linked Immunosorbent Assays (ELISAs), have varied little over the same time period. The techniques used in today's lab still employ laborious, time-consuming procedures with limited dynamic ranges.

Fortunately, a novel alternative for quantification of secreted IgG antibodies is now available with the Human and Mouse Guava RapidQuant™ Assays. These simple, no-wash, bead-based assays use less than ten microliters of supernatant, minimize sample handling, and deliver results comparable to traditional ELISAs.

### Simple, mix-and-read procedure.

RapidQuant Assays are mix-and-read procedures, which are easier, quicker, and have far fewer hands-on steps compared to traditional ELISAs. The total assay time is typically less than three hours, and the procedures are refreshingly straightforward. Simply add hybridoma supernatant, most often without dilution, directly to RapidQuant™ anti-IgG beads in microplates for capture. Then, add the antibody detector and incubate. Finally, add buffer to the resulting mixture and read on a Guava EasyCyte™ System. The assay procedure allows for easy quantification of antibody in supernatant without tedious dilution procedures or wash steps.

	RapidQuant Assay	Traditional ELISA
Pre-assay dilution	No*	Yes
Pipet steps	4	≥10
No. washes	0	≥8
Assay-specific analysis	Yes	No
Ave. time	<3 hrs.	≥6 hrs.

\*Under typical assay conditions

### Minimal sample handling.

To quantify the concentration of an unknown sample accurately, that concentration must be within the linear portion of a standard curve.

Figure 1 shows standard curves from a RapidQuant Human IgG Assay and an ELISA. While both methods can generate accurate predictions, ELISAs are generally more labor-intensive since the hybridoma sample must often be diluted numerous times before it is within the linear portion of a standard curve. With RapidQuant Assays, you can generally avoid this step because the dynamic range of the assays spans the typical concentration range for hybridoma supernatants.

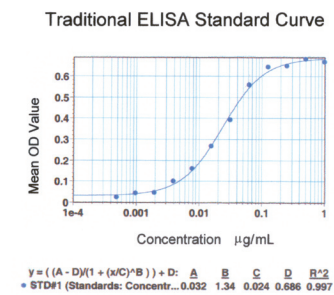
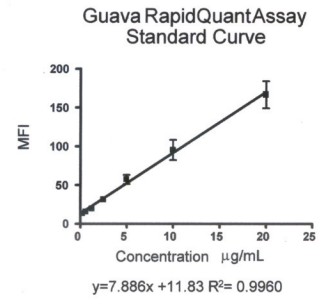


Figure 1: Typical standard curves for RapidQuant Assay & traditional ELISA. RapidQuant Human Assay has a dynamic linear range between 0.5 – 20 µg/mL.

### Results comparable to traditional ELISA.

RapidQuant Assays deliver results that are comparable to traditional ELISAs. Figure 2 shows that the RapidQuant Human IgG Assay has virtually identical measurement power for predicting antibody concentration in supernatants as an in-house ELISA, courtesy of PDL BioPharma Inc.<sup>1</sup>

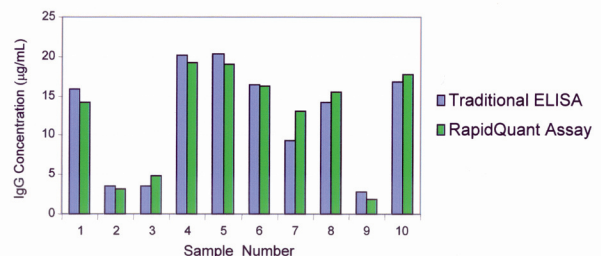


Figure 2: RapidQuant Human and traditional ELISAs have virtually identical measurement power for predicting antibody concentration in the supernatants.

## Assay-specific analysis software.

In addition to straightforward robust protocols the Guava RapidQuant IgG Assays are now even easier and more powerful with the availability of new assay-specific software. Guava RapidQuant™ Software provides a simplified, easy-to-use interface in converting the cytometry based data into IgG concentration data. Assay-specific features are default-selected yet still give users the flexibility to change parameters if desired.

Key features of the software include:

- **Simple acquisition**, users can load WorkEdit files with selected parameters.
- **Easy analysis**, users can use color-coding for assignment of wells as standards, controls, and unknowns.
- **Straightforward assignment**, users simply choose standard values from a selected standard set.
- **Automatic plotting**, software calculates standard curve and predicts unknown concentrations automatically.
- **“Cut-and-paste” standard curve**, just copy standard curves directly into software programs for presentation or other purposes.
- **Flexible report generation**, users can export, print, or save reports with standard curve, plate map, and concentration table.
- **Fast data export**, just click a button to export summary data as a csv file.

## Ordering information.

Part ID	Description	Qty
4500-0340	Guava RapidQuant™ IgG Reagent Kit (human)	1
4500-0350	Guava RapidQuant™ IgG Reagent Kit (mouse)	1
0500-2310	Guava RapidQuant™ Software	1

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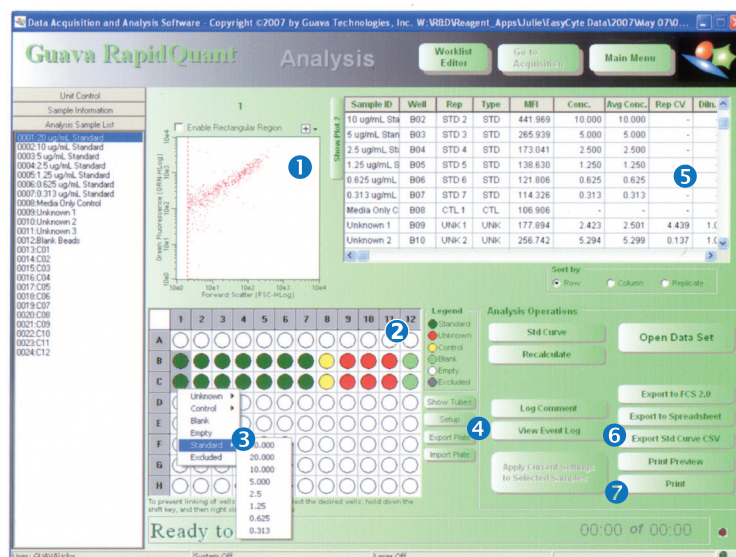


Figure 3: RapidQuant Assay software is simple and powerful. In Analysis Mode you can: **1)** View sample dot-plot, **2)** Label samples as standards, unknowns, controls, & blanks, **3)** Assign standards from a drop-down menu, **4)** Change list for standards or units **5)** Calculate concentration, averages, & %CV, **6)** Export summary file with MFI & concentration, and **7)** Print data with standard curve and unknown concentrations.

## Conclusion.

RapidQuant IgG Reagent Kits, together with RapidQuant Assay-Specific Analysis Software, offer users an unparalleled mix of simplicity and performance. The no-wash, easy-to-perform protocols and user-friendly analysis environment empower researchers with novel, time-saving strategies for selecting optimal clones during hybridoma screening. The quantification processes are straightforward, with easy set-up, minimal sample handling, simplified data acquisition, and automatic calculations. In addition, numerous data management options offer flexibility and customization. The end result is an ideal assay for hybridoma screening that combines the reliability of flow cytometry, a tried-and-true technology, with the convenience of a plate-based format.

Call Guava Technologies today for a complimentary demonstration of this novel, powerful assay.

## References.

1. Jia, A., et al., Rapid and accurate quantitation of human antibody production using the Guava RapidQuant™ Assay, *Antibody Engineering & Immunotherapeutics for the 21st Century*, December 2006.



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