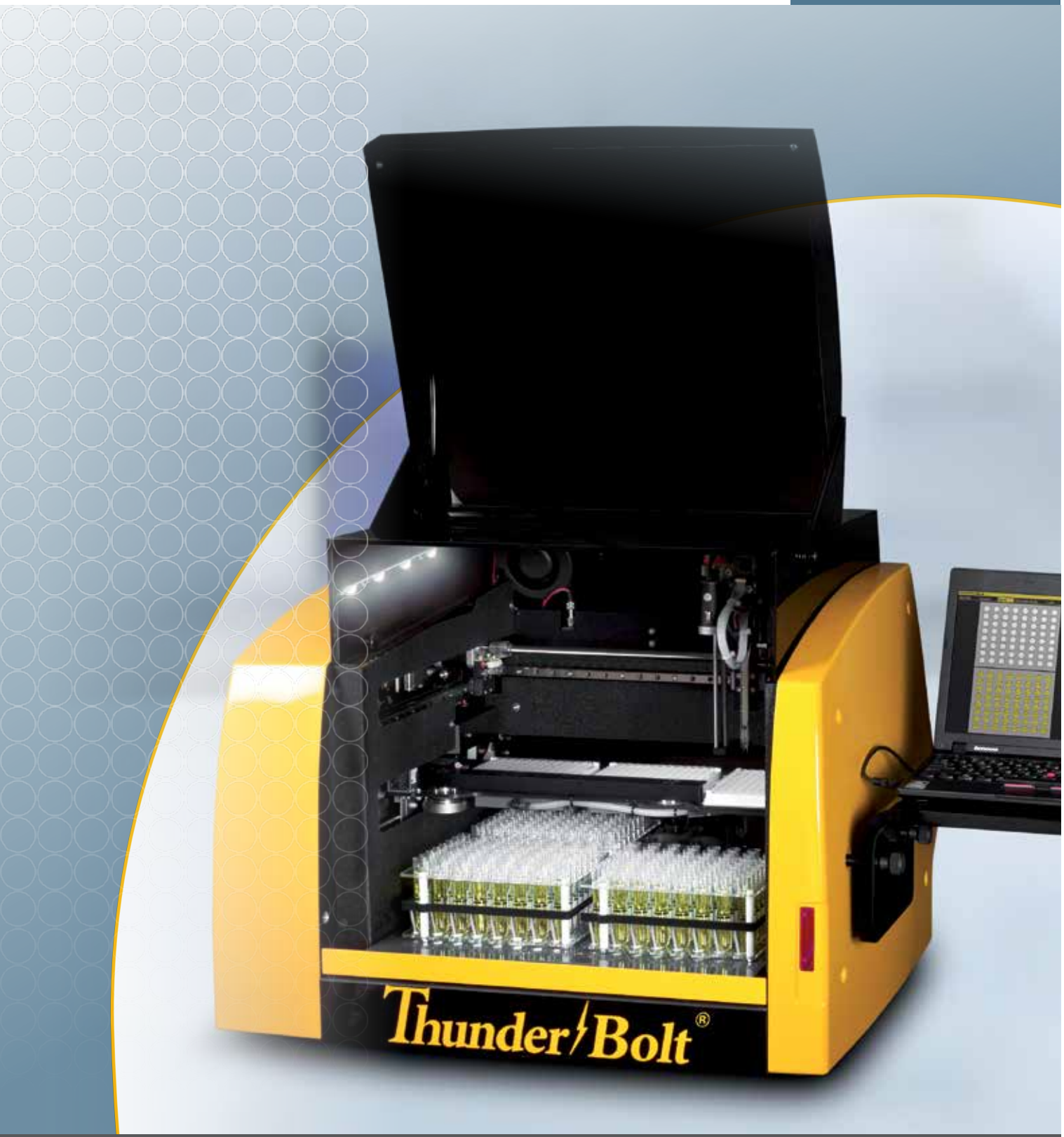
R-Biopharm AG

ThunderBolt® automation for RIDASCREEN® Gliadin Art. No. R7001

Product information



R-Biopharm – for the safety of your analysis.

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General

The aim of this report is to compare the manual handling with the automated analysis of food samples using the RIDASCREEN® Gliadin (Art. No. R7001). The automated version uses the same test kit components as the version by hand. The pipetting volumes (100 µl) and the incubation times are the same in both cases. For the automate version an optimized washing procedure is carried out to ensure reliable and accurate results. All assays have been carried out at room temperature.

The manual procedure of the test kit is usually carried out with the following equipment:

- Pipettes for pipetting the samples and standards
- Multistepper for pipetting conjugate and stop solution
- 8 channel pipette for pipetting the wash buffer
- Microtiter plate photometer (e.g. Biotek® ELX 800™) measuring at 450 nm
- RIDASOFT® Win.NET for calculation of results

The ThunderBolt® automate is a two-plate ELISA processor including fully automated pipetting with an integrated software to calculate results. The absorbance is measured against air at 450 nm. The total working time for a microtiter plate is approx. 120 minutes. It is important to make sure that air bubbles in the wells do not impede the absorption readings.

The test run is valid within a temperature range of 20 - 28 °C (68 - 82 °F) on automated systems.
Due to the fact that ThunderBolt® is not able to cool, the user has to make sure that the conditions are within the specification (20 - 28 degrees).

The following report is available from R-Biopharm:

- Validation report RIDASCREEN® Gliadin (information on performance, sensitivity, variance, reproducibility for manual use

Test principle

The basis of the sandwich ELISA is an antigen-antibody reaction. Detailed information is contained in the test kit insert.

Specificity

The monoclonal antibody R5 reacts with the gliadin-fractions from wheat and corresponding prolamins from rye and barley.
The result is read from the curve in mg/kg (ppm) gliadin. A typical standard curve for manual pipetting is shown in the quality assurance certificate (insert in the test kit). The result is expressed as mg/kg (ppm) gliadin. To create the calibration curve a cubic spline function is recommended.

Due to the multitude of food types, matrix effects cannot be excluded. To ensure an accurate result spike experiments are recommended.

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Homogeneity testing

To validate the ThunderBolt® automate homogeneity testing of sample pipetting and assay procedure was carried out. The same sample extract was pipetted to all of the 96 wells of three antibody coated microtiter plates from one batch following the test procedure using the optimized automate settings of the ThunderBolt® for gliadin analysis. Afterwards the standard deviations (SD) and the coefficients of variation (CV) of the measured optical density (OD) were calculated (n=96) for each microtiter plate. The coefficients of variation are shown in table 1 (see table 1).

Table 1: The same sample extract was pipetted to three coated microtiter plates. The Standard deviation SD and Coefficient of variation CV (%) were calculated for each plate (n = 96 single values).

Plate	Mean optical density	Standard deviation (SD)	CV* (%)
Microtiter plate 1	1.078	0.045	4.2
Microtiter plate 2	0.917	0.06	6.5
Microtiter plate 3	1.024	0.061	5.9

* CV = coefficient of variation

Sensitivity

Limit of Detection

The LOD of the test kit has been determined manually (please refer to the manual validation report of this assay). The Field Service Unit of R-Biopharm verifies only the deviation at low concentrations. To do so 3 different negative samples are run (n = 84) and the coefficient of variation of the OD is calculated.

Table 2: Determination of the Limit of Detection (LOD).

Sample	Standard 1 n=2 (OD)	Standard 2 n=2 (OD)	Mean of samples (OD)	Standard deviation (SD)	B/B max (%)	CV (%)
Cookie	0.143	0.26	0.099	0.005	5	5.2
Oat meal	0.127	0.273	0.131	0.008	6.7	6.3
Rice flour	0.126	0.286	0.147	0.011	6.6	7.4
Mean						6.3

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Limit of Quantification

The **Limit of Quantification** or the lowest concentration that can be determined in a sample with acceptable precision (repeatability) and accuracy under the stated conditions of the test was proven to be 2.5 ppm gliadin 2 (dilution factor of 500 included).

The LOQ is proven by showing the range of samples spiked at the level of standard 2 (2.5 mg/kg gliadin) with 84 replicates.

Table 3: Proof of the Limit of Quantification (LOQ) with spiked samples in comparison to standard 2.

Sample	Mean OD S2	Mean OD sample	SD sample	Concentration extrapolated (mg/kg)	B/Bmax (%)	CV (%)
Cookie	0.183	0.225	0.018	3.16	12.0	7.9
Oat meal	0.163	0.195	0.022	2.92	11.8	11.2
Rice flour	0.162	0.234	0.026	3.50	13.2	11.0

Precision

Precision (scatter of replicate readings around their mean value)

To determine the reproducibility and repeatability of the automated version the intra-assay and inter-assay variances were calculated.

Intra-Assay Variation (repeatability)

Within run variation was calculated by measuring the standards in one assay run (n = 10). The within assay variation of the standard curve is shown in table 4. The average intra-assay variation of spiked samples is shown in table 5.

Table 4: Coefficients of variation (CV) for the gliadin standard curve within one assay (n = 10).

Plate	Mean optical density (OD)	Standard deviation (OD)	CV (%)
Standard 1	0.038	0.002	6.1
Standard 2	0.208	0.014	7.0
Standard 3	0.428	0.020	4.6
Standard 4	0.835	0.052	6.3
Standard 5	1.533	0.135	8.8
Mean CV			6.5

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Table 5: Determination of the Intra-Assay Variation (repeatability) of spiked samples (n=8).

Sample	Target protein concentration (mg/kg)	Measured concentration (mg/kg)	CV (%)	Recovery (%)
Custard powder	0	LOQ	6.7	
Corn flour	11.8	13	4.5	110
Corn flour	20.8	29.7	2.7	142
Rice flour	8.5	7.5	4	88
Mean value			4.5	113

Inter-Assay Variation (intra-lab reproducibility)

Between run variation was determined by repeated measurements of the standard curves from different test kits of one production batch at 5 different days. In each run duplicates of samples and standards have been measured. The mean between assay variance for the RIDASCREEN® Gliadin is shown in table 6.

The inter-assay variance of one negative and two spiked food samples is shown in table 7.

Table 6: Coefficients of variation (CV) for the gliadin standard curve between different assays (n=5).

Plate	Mean optical density (OD)	Standard deviation (OD)	CV (%)
Standard 1	0.092	0.030	33.0
Standard 2	0.269	0.036	13.3
Standard 3	0.462	0.021	4.5
Standard 4	0.819	0.023	2.9
Standard 5	1.422	0.079	5.5
Mean CV			10.8

Table 7: Determination of the Inter-Assay Variation (reproducibility) of spiked samples.

Sample	Target protein concentration (mg/kg)	Measured concentration (mg/kg)	Standard deviation (mg/kg)	CV (%)	Recovery (%)
Corn flour	8.5	7.7	0.272	3.5	91
Corn flour	11.8	17.7	4.3	24	136
Rice flour	0	< LOQ	0.09	8.5	< LOQ
Mean value					114

* CV = coefficient of variation

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Comparison of automation and manual pipetting for different samples

Different gliadin-free samples were spiked and additional gliadin containing samples were chosen to be analyzed with the ThunderBolt® after extraction. The results obtained by automate correlate well with the manual use. The recovery of the ThunderBolt (Warenzeichen nicht vergessen) ranged from 75 - 175 %. Usually, the ELISA recovery of samples using automates should be between 60 - 140 %.

Table 8: Comparison between manual and automated analysis using the same incubation time.

Sample	Extraction method	Final dilution	Target protein concentration (mg/kg)	Automate recovery (%)	Manual recovery (%)	Difference (in %)
Corn flour	Cocktail	500	8.5	82	85	3
Corn flour	Ethanol	500	8.5	89	70	19
Corn flour	Ethanol	1.000	8.5	71	73	2
Cornmeal	Cocktail	500	0.0			
Corn Snack	Cocktail	500	23.8	96	75	21
Corn Snack	Cocktail	2.000	53.2	114	97	17
Corn snack	Cocktail	4.000	53.2	115	87	28
Cornmeal	Ethanol	500	11.8	127	98	29
Cornmeal II	Cocktail	500	20.8	175	127	48
Rice flour	Ethanol	500	0.0			
Rice flour	Ethanol	1.000	0.0			
Sausage	Cocktail	500	0.0			
Mean				109	89	

On ‘Board Stability’ of the test

The on ‘Board Stability’ of the test is routinely checked by R-Biopharm‘s field service unit during validation.

The procedure for testing ‘On Board’ stability is as follows:

- A new test kit is opened and samples are prepared according to the instructions in the test kit insert. The assay is then run on the automate without any delay.
- After the run all reagents and samples remain in the automate for 4 hours. After 4 hours the automate carries out the analysis.
- After the 4 hour run all reagents and samples remain in the automate for another 20 hours. After 20 hours the automate carries out the analysis. In addition to this run a fresh kit and fresh samples are analyzed as well.

The aim of this testing is to check the ‘onboard stability’ over a period of 24 hours without significant impact on the analysis.

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Carry over testing

In automates using only one needle for pipetting a carry-over effect may occur. This means that high positive samples could contaminate following negative samples due to insufficient rinsing steps of the needle.
This is why the R-Biopharm 'Field Service Unit' analyses high positive samples following negative samples for each assay to demonstrate the reliable and robust rinsing of the needle.

Conclusion

It could be shown that the settings of the optimized ThunderBolt® technical protocol lead to a good performance. The results are comparable to the manual handling. RIDASCREEN® Gliadin can be run on a ThunderBolt® automate.

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