

# Protein A ELISA Kit Stability Report

## Technical Brief

### 1. Scope

The purpose of the study was to provide data to support the current 12-month shelf life date.

This report also provides data that demonstrates the stability of the kit following exposure temperature excursions as might be experienced during transit and handling (stress testing).

### 2. Introduction

Repligen manufactures a Protein A ELISA Kit for the detection of residual protein A in process solutions. The kit is assigned an expiration date of one year from the date of kit assembly.

The Protein A ELISA Kit is based on a 96-well plate coated with chicken anti-rPA polyclonal antibody. The kit also contains the following reagents:

- Reagent A – sample diluent
- Reagent B – recombinant Protein A (rPA) standard solution
- Reagent C – rabbit anti-protein A: Biotin probe
- Reagent D – streptavidin:HRP conjugate
- Reagent E – tetramethylbenzidine peroxidase substrate
- Dehydrated PBS packets

The assay requires the construction of two 7-point standard curves (6 strips each). The reference curve is made using the reagents and plate strips from the reference (new, unstressed and QA released) kit. The second curve is made using reagents and plate from the ELISA kit being tested (either a stressed kit or a kit that has been put aside for stability testing).

The standard curve is constructed using the rProteinA standard that is included in the kit. The standard is serially diluted to 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 ng/mL. A zero standard is also included (diluent only). The plate containing the diluted samples is then developed as per the standard kit protocol. Both the 'test' and 'reference' kits are evaluated based on the standard curve. The acceptance criteria are shown below in table 1.

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Table 1: Protein A ELISA Kit release criteria.

Performance Criterion	Specification
Correlation coefficient ( $R^2$ ) for the standard curve with linear regression	$\geq 0.985$
Correlation coefficient ( $R^2$ ) for four parameter logistic standard curve fit	$\geq 0.990$
OD value for 1.6 ng/mL Standard	0.900 OD or greater at 450 nm
OD value for 0 ng/mL Standard	0.150 OD or lower at 450 nm
CV of 0 ng/mL Standards	Less than 18% with no more than two masked outliers per sextuplet data set
CV of other Standard Curve points	Less than 12% with no more than two masked outliers per sextuplet data set
Hot Wells	No more than four total (two per sextuplet data set)

### 2.1. Study design

Shelf Life Testing: Kits are taken from inventory at release, and are stored according to the instructions in the User guide. At each specified time point, a kit is removed from storage and tested as per the current QC release protocol. The acceptance criteria are that the kit must meet release criteria at each stability time point.

Three test time points are scheduled per kit (in addition to the QC release test), time zero (release testing), 9 months and then 15 months post manufacture.

Temperature Excursion Testing: This stress testing is designed to investigate the immediate impact of short term adverse storage conditions on kit performance.

## 3. Procedures and Results

### 3.1. Temperature Excursion Stress Testing

Kits were exposed to high temperature and low temperature stress. The objective of this part of the study was to evaluate the impact of short-term temperature excursions, e.g. during shipping deviations, or due to inappropriate handling. The data may be used to support customer inquiries, but will not be used to make any claims of kit stability.

#### 3.1.1. High temperature stress:

Kits were removed from 2-8°C storage, and a reagent B vial was removed from -20°C storage and placed into the kit. The assembled kits were placed in a 37°C incubator for stress testing, and then tested as per QCP-1009

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### 3.1.2. Low temperature stress:

Assembled kits (including reagent B) were placed in a -20°C storage unit. The kits were then removed from the incubator, allowed to thaw, and then tested as per QCP-1009.

### 3.2. Temperature Excursion Stress Testing Results

The results of the stress testing are summarized below:

Table 2: Stress test data

Kit Lot	Stress temp	Stress time	Result
RN071885	37°C	26 hr 25 min	Pass
		49 hr 15 min	Pass
RN080095	37°C	138 hr 50 min	Pass
RN081151	37°C	24 hr 5 min	Pass
		52 hr 0 min	Pass
RN071545	-20°C	166 days	Pass
RN081726	-20°C	226 days	Pass

The data indicate that temperature excursions are unlikely to impact the performance of the ELISA Kits. Exposure to higher than normal temperature does not adversely impact the performance of the kit. The data also show that if kits are exposed to freezing temperatures during transit, there is no impact on performance

### 3.3. Stability study procedure

At each specified time point, one kit and corresponding reagent B vial were removed from storage, equilibrated to room temperature, and then tested per Repligen's standard QC release testing protocol.

#### 3.3.1. Shelf Life - Stability data: 9 month time point

Results for the seventeen (17) kits tested at the 9 month time point are summarized in table 3.

#### 3.3.2. Shelf Life - Stability data: 15 month time point:

Results for fourteen (14) kits tested at the 15 month time point are summarized in table 3.

### 3.4. Shelf Life Testing Results

The data in table 3, supports Repligen's shelf life claim for the protein A ELISA Kits of at least 12 months.

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### 4. Conclusion

Stress testing demonstrated that the kit can be frozen without impacting kit performance. Furthermore, the kit may be exposed to temperatures up to 37°C for up to 5 days without impacting performance.

The data in this report show that the kits (17) tested after 9 months and kits (14) tested beyond the 12-month expiration period after 15 months continued to meet advertised performance specifications and QC release criteria

The conclusion of this report is that the Protein A ELISA Kits will continue to perform as specified up to its 12 month expiration date.

Note: The data in this report is derived from internal Quality Document “ELISA Kit Stability Study Interim Validation Report following protocol VMP061101” and is summarized in report number R-100805 revision 1. Both the validation protocol and interim report are available for review at Repligen as part of an on site audit.

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Table 3: Protein A ELISA kit stability data through 15 months.

Kit lot#	Coating Antibody Lot #	Release test (time = zero)				9 months				15 months			
		Assembly Date	Signal	Linear R <sup>2</sup> (≥0.985)	Test Date	Age (mo)	Signal (0.900/0.150)	Linear R <sup>2</sup> (≥0.985)	Test Date	Age (mo)	Signal (0.900/0.150)	Linear R <sup>2</sup> (≥0.985)	
RND61641	#4	9/25/2006	1.079/0.111	0.998	6/25/2007	9.1	1.125/0.074	0.999	No Test Result	No Test Result			
RND61887	#4	12/11/2006	0.946/0.033	1.000		No Test Result			3/6/2008	15.0	1.000/0.029	1.000	
RND62143	#4	1/26/2007	1.458/0.084	0.988	11/12/2007	9.7	1.375/0.092	0.99	5/7/2008	15.6	1.069/0.055	0.996	
RND70113	#4	3/15/2007	0.973/0.114	0.997	12/28/2007	9.6	1.162/0.092	0.998	6/12/2008	15.2	0.902/0.051	0.997	
RND70576	#4	4/6/2007	1.293/0.099	0.999	2/29/2008	11.0	1.277/0.139	0.998	6/27/2008	14.9	0.978/0.059	0.999	
RND70708	#4	5/11/2007	1.016/0.098	0.997	2/22/2008	9.6	1.023/0.065	0.997	7/31/2008	14.9	1.065/0.053	0.992	
RND71885	#4	7/27/2007	1.272/0.110	0.998	4/30/2008	9.3	1.186/0.072	0.999	10/27/2008	15.3	1.736/0.093	0.995	
RND73099	#4	10/3/2007	1.200/0.130	0.997	6/27/2008	8.9	0.956/0.072	0.999	1/6/2009	15.4	1.332/0.070	0.999	
RND74627	#7	2/15/2008	1.389/0.099	0.999	11/17/2008	9.2	1.193/0.065	0.995	5/19/2009	15.3	1.285/0.083	0.995	
RND80095	#7	3/5/2008	1.340/0.096	0.996	12/3/2008	9.1	1.068/0.043	0.997	6/4/2009	15.2	1.433/0.063	0.998	
RND81151	#7	6/4/2008	1.663/0.117	0.995	3/5/2009	9.1	1.419/0.070	0.996	9/15/2009	15.6	1.652/0.060	0.993	
RND81726	#7	7/25/2008	1.451/0.092	0.994	4/27/2009	9.2	1.538/0.082	0.997	10/21/2009	15.1	1.410/0.066	0.999	
RND82504	#7	9/9/2008	1.375/0.091	0.992	6/12/2009	9.2	1.593/0.060	0.994	12/10/2009	15.2	1.416/0.068	0.997	
RND82716	#7	11/18/2008	1.712/0.088	0.997	8/13/2009	8.9	1.056/0.048	0.995	2/19/2010	15.3	1.307/0.073	0.996	
RND83123	#7	2/24/2009	1.876/0.085	0.993	11/23/2009	9.1	1.195/0.057	0.997	5/25/2010	15.2	1.530/0.057	0.998	
RND83832	#7	4/22/2009	1.514/0.078	0.996	1/25/2010	9.3	1.210/0.071	0.996					
RND90207	#7	6/11/2009	1.773/0.108	0.992	3/15/2010	9.2	1.541/0.092	0.993					
RND90286	#7	8/11/2009	1.556/0.081	0.995	5/10/2010	9.1	1.555/0.084	0.998					