AAV9 Titration ELISA Kit (Fast)

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：AAV-A009H

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

INTENDED USE

AAV9 Titration ELISA Kit (Fast) was developed for the detection and quantitative determination of AAV9 capsid titration in AAV gene therapy product preparation processing. It is intended for research use only (RUO).

BACKGROUND

The adeno-associated virus (AAV) is a small (25 nm), non-enveloped virus of the parvoviridae family, including 12 different AAV serotypes. In the parvoviridae family it belongs to the genus depend on parvovirus, because it needs the presence of a helper virus for replication and assembly. The icosahedral AAV capsid composed of the capsid proteins VP1, VP2 and VP3 contains a linear, single-stranded DNA genome of 4.7 kb.

To support the development of gene therapy, ACROBiosystems independently developed AAV9 Titration ELISA Kit (Fast) via rigorous methodological validation, which is used for detection of AAV9 capsid titration in samples.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of AAV9 capsid by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-AAV9 Antibody. Firstly, add the standard samples provided in the kit and your samples to the plate, incubate and wash the wells. Then add the HRP-Anti-AAV9 Antibody to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor the solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of AAV9 capsid bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not use reagents past their expiration date.
3. Do not mix or substitute reagents with those from other kits or other lot number kits.
4. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
5. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

washing technique, reaction time or temperature, and kit storage.

6. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
AAV09H-C01	Pre-coated Anti-AAV9 Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
AAV09H-C02	AAV9 Standard	3.80E+11 capsids	Powder	2-8℃	-70℃
AAV09H-C03	HRP-Anti-AAV9 Antibody	20 μg	Powder	2-8℃, avoid light	-70℃, avoid light
AAV09H-C04	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
AAV09H-C05	2×Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
AAV09H-C06	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
AAV09H-C07	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

SRORAGE

1. Unopened kit should be stored at 2℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL, 10mL;

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

2. According to Table 2, prepare the provided lyophilized product into a storage solution, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. AAV09H-C02 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 25 μL. AAV09H-C03 is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5μg.

Note: *Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted Buffer Vol.
AAV09H-C02	AAV9 Standard	3.80E+11 capsids	3.80E+12 capsids/mL	100 μL 1×PBS
AAV09H-C03	HRP-Anti-AAV9 Antibody	20 μg	200 μg/mL	100 μL Water

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

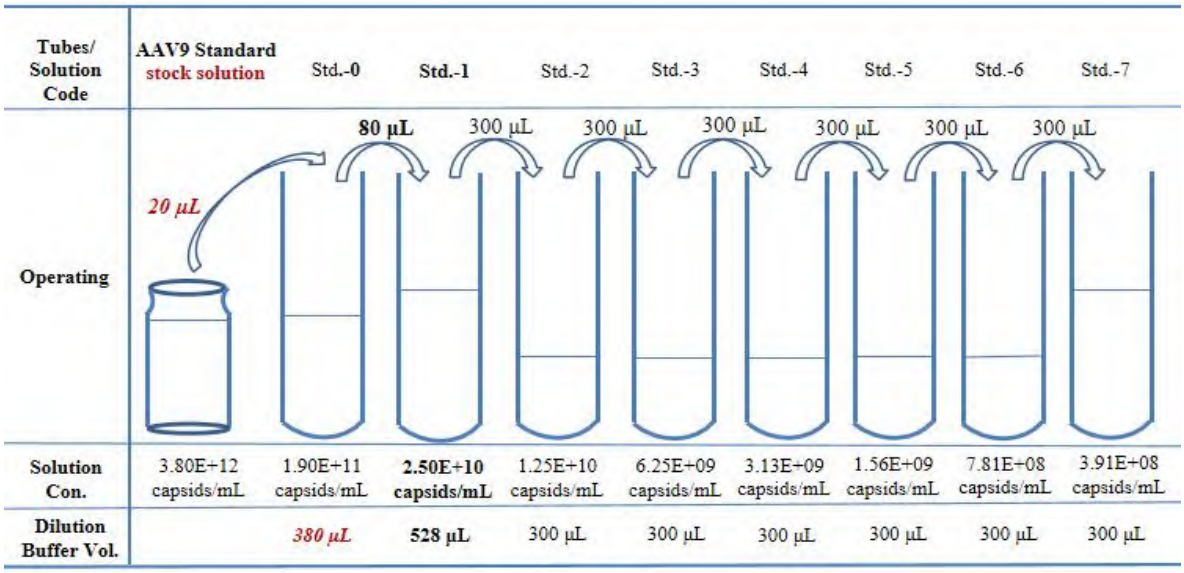
1.3 Preparation of HRP-Anti-AAV9 Antibody working fluid:

Dilute HRP-Anti-AAV9 Antibody to 0.02 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

US and Canada: Tel: +1 800-810-0816 | Web: http://www.acrobiosystems.com
Asia and Pacific: Tel: +86 400-682-2521 | E-mail: order@acrobiosystems.com

2. Preparation of Standard Curve

The concentration of the reconstituted AAV9 Calibrator (AAV09H-C02) is 3.80E+12 capsids/mL， prepare (Std.-0) by diluting 20 µL the reconstituted AAV9 Calibrator into 380 µL Sample Dilution Buffer, mix gently well. Then prepare the highest concentration of standard curve, Std.-1 (2.50E+10 capsids/mL), by diluting 80 µL Std.-0 into 528 µL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add HRP-Anti-AAV9 Antibody

For all wells, add 100 µL HRP-Anti-AAV9 Antibody (dilute to 0.02 µg/mL) working solution. Please prepare it for

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 hour.

8. Washing

Repeat step 5.

9. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 min, avoid light.

10. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

11. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

Note*: To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\,nm}$.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).

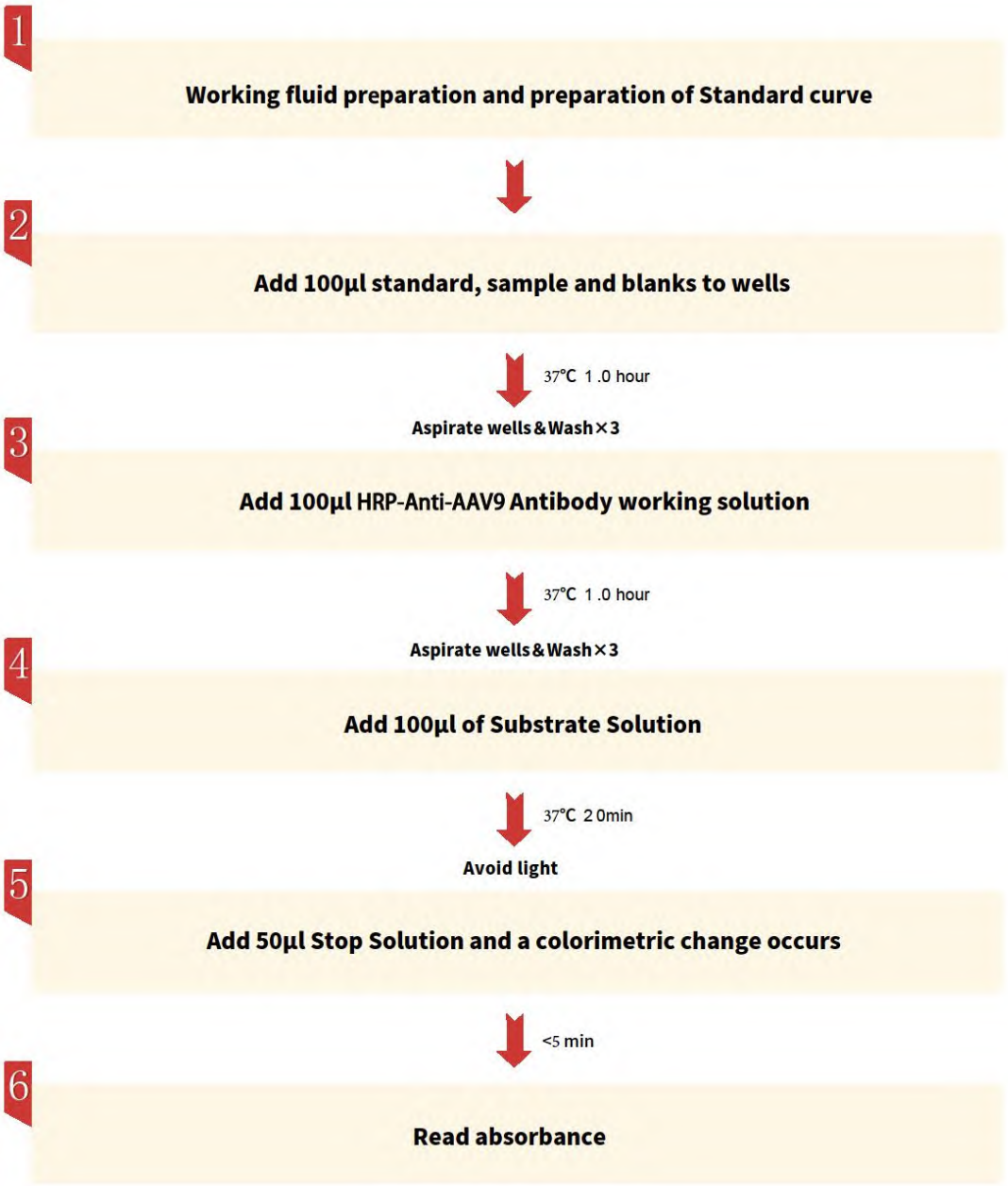
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

4. Detection range: 3.91E+08 capsids/mL-2.50E+10 capsids/mL. If the OD value of the sample to be tested is higher than 2.50E+10 capsids/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 3.91E+08 capsids/mL, the sample should be reported.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

QUICK GUILD

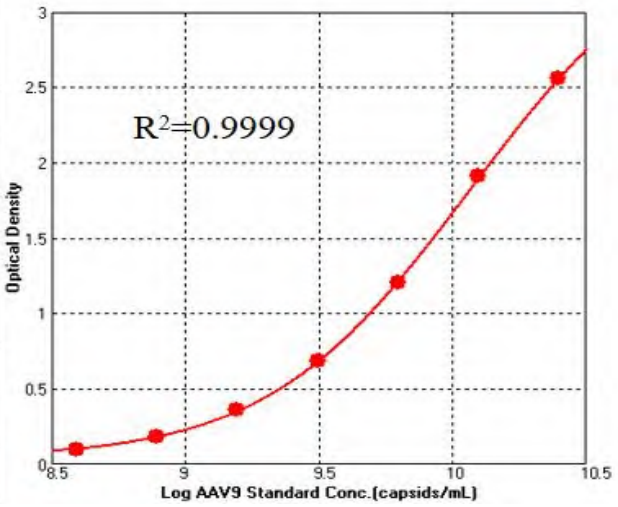


US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (capsids/mL)	O.D.-1	O.D.-2	Average	Corrected
2.50E+10	2.573	2.571	2.572	2.559
1.25E+10	1.942	1.909	1.926	1.913
6.25E+09	1.170	1.264	1.217	1.204
3.13E+09	0.678	0.712	0.695	0.682
1.56E+09	0.369	0.389	0.379	0.366
7.81E+08	0.195	0.203	0.199	0.186
3.91E+08	0.105	0.108	0.107	0.094
0	0.013	0.013	0.013	/



SENSITIVITY

The minimum detectable concentration of AAV9 is 1.69E+07 capsids/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (capsids/mL)	1.90E+10	5.01E+09	7.70E+08	1.88E+10	5.04E+09	7.96E+08
SD	1.11E+09	2.64E+08	3.59E+07	3.21E+08	1.31E+08	2.48E+07

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

CV (%)	5.9	5.3	4.7	1.7	2.6	3.1

Note: *The example data is for reference only.*

RECOVERY

Three AAV9 with different concentrations were tested to calculate the recovery rate.

Sample(n=5)	Average Recovery %	Range %
High	91.7	86.7-101.4
Middle	96.8	90.5-108.3
Low	102.4	100.1-106.7

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of AAV9 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)
1:2	Average Recovery (%)	92.2	104.7
	Range (%)	88.7-95.6	97.5-116.3
1:4	Average Recovery (%)	94.7	107.7
	Range (%)	90.7-97.9	101.7-111.2
1:8	Average Recovery (%)	98.3	106.4
	Range (%)	94.0-103.3	99.5-118.1
1:16	Average Recovery (%)	103.4	106.3
	Range (%)	90.8-110.9	95.2-116.2

Note: *The example data is for reference only.*

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

SPECIFICITY

This assay recognizes AAV9 specifically. No cross-reactivity was observed when this kit was used to analyze the following AAV serotypes.

Specificity		
AAV1	AAV2	AAV3
AAV5	AAV6	AAV8
AAV dj		

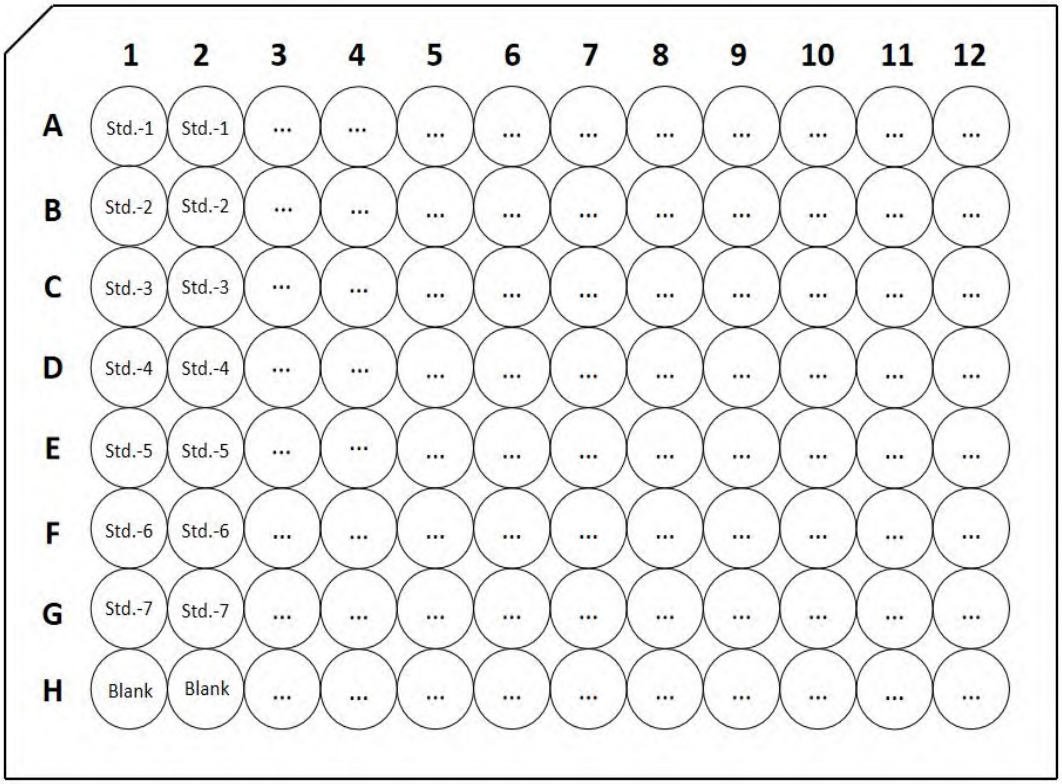
INTERFERING SUBSTANCES

Analysis of matrix effects of different additives on the AAV9 Titration ELISA.

Additive/Matrix	Tolerated concentration
Pluronic F-68	0.05%
$MgCl_2$	10 mM
Triton X-100	0.5%
Tween20	0.5%
EDTA	10 mM
NaCl	0.5 M

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

PLATE LAYOUT



Note: *Blank is a Blank Dilution Buffer hole.*

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com