Express™ ELISA Kit

Technical Manual No. 0239 **Version 20070325**

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I. DESCRIPTION

The GenScript Express™ ELISA kit is designed to detect and quantitate antigen-specific antibodies in serums or other samples using a proprietary (patent pending) one-hour indirect ELISA procedure. This kit allows user to perform a complete ELISA from antigen coating to signal development in just one hour. The one-hour Express™ ELISA procedure is contrasted with a classical five-hour ELISA procedure at right (Figure 1).

Coat the plate with an antigen (protein or peptide) in the Coating Buffer for five minutes. Then block the plate with Pretreat Solution for five minutes so that it will be ready for antigen-specific antibody binding. After the 30-minute binding phase, treat the plate with the ELISA solution included in the kit for 20 minutes. No secondary antibody is needed. The plate can then be developed with the One-Solution Microwell TMB solution included in the kit.

The kit contains all necessary reagents and buffers for performing indirect ELISA using ten 96-well plates.

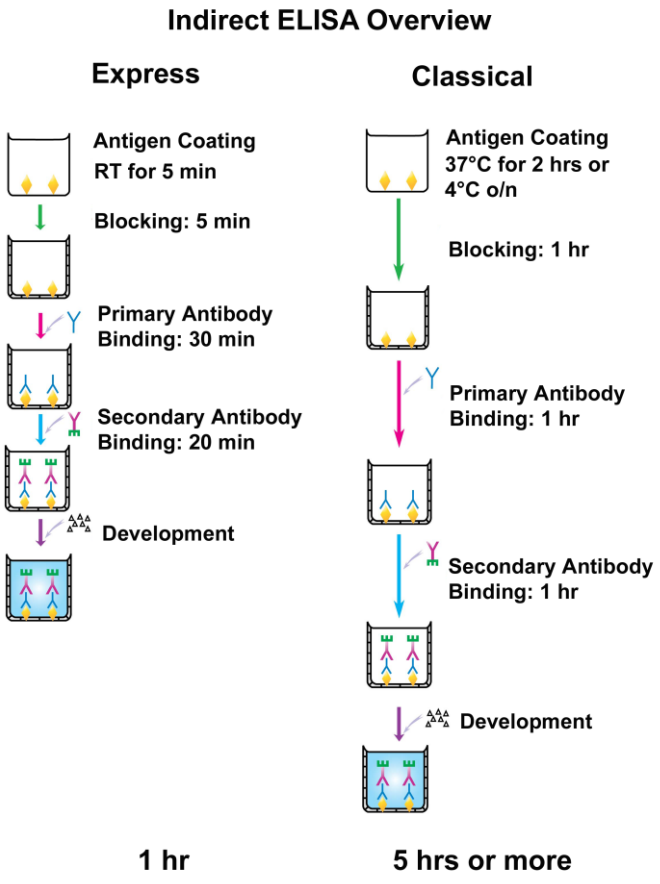


Figure 1. Overview of Indirect ELISA Procedures

II. KIT CONTENTS

Three different kinds of Express™ ELISA Kits are available. These are for use with rabbit (L00251), mouse (L00252), and goat (L00253) primary antiserum or antibodies, respectively. Each kit contains enough reagents for ten 96-well plates.

Warning: Wear gloves when handling the reagents. Some of them are corrosive!

Kit Components	L00251 (Rabbit) For 10 Plates	L00252 (Mouse) For 10 Plates	L00253 (Goat) For 10 Plates
2X Coating Buffer	125 ml	125 ml	125 ml
Pretreat A Solution	125 ml	125 ml	125 ml
Pretreat B Solution	125 ml	125 ml	125 ml
Dilution Solution	2 X 125 ml	2 X 125 ml	2 X 125 ml
ELISA Solution	125 ml	125 ml	125 ml
20X Wash Solution	125 ml	125 ml	125 ml
One-Solution Microwell TMB	110 ml	110 ml	110 ml
Stop Solution	125 ml	125 ml	125 ml
Protocol	1	1	1

Materials not provided:

ELISA plates are not provided. Limited tests at GenScript show that the kits are compatible with any commercially available ELISA plates.

III. RELATED PRODUCTS

- 2X Coating Buffer M01018
- One-Solution Microwell TMB M00078
- Stop Solution M01017
- 20X Wash Solution M01016
- Pretreat Solution (A + B) M01019

IV. KEY FEATURES

- ♣ Quick: This kit cuts indirect ELISA time down to one hour.
- ♣ High sensitivity: The kit's sensitivity is the same as or better than that of the classical procedure.
- ♣ Reproducible results: The kit produces highly reproducible results.
- ♣ Less optimization: The Express™ ELISA needs far less optimization than the classical ELISA method.

V. STORAGE

Store the kit at 4°C. It will remain stable for six months. **Do not freeze the kit or any of its components**.

VI. EXPRESS™ ELISA PROTOCOL

This procedure is optimized for one 96-well plate. The volumes of the reagents can be scaled up or down according to the numbers of the plates used.

Before use, prepare the following:

Dilute 12 ml of 20X Wash Solution with 228 ml of distilled or filtered water to make 240 ml of 1X Wash Solution. If any precipitate forms in the 20X Wash Solution during storage, incubate the bottle in warm or hot water bath (up to 50 °C) with occasional mixing until all the precipitate disappears. Dilute the buffer with ddH_2O to 1X and store it at 4 °C.

Express™ ELISA procedure:

1. **Antigen coating.** First, dilute the antigen with ddH_2O (Do not use any buffer because the buffer could affect the coating efficiency.) to about 20 µg/ml. Then dilute the antigen with 2X Coating Buffer to the appropriate concentration (1-10 µg/ml). Add 100 µl per well of the antigen solution and incubate at room temperature (about 25°C) for 5 minutes. **The antigen solution in Coating Buffer should be used within 30 min.** After incubation, remove and discard the coating solution. No wash is necessary; proceed directly to the next step.
2. **Blocking plate.** Add 200 µl/well of freshly made Pretreat Solution, made by mixing equal volumes of Pretreat A with Pretreat B, and incubate at room temperature for 5 minutes. **The freshly made Pretreat Solution should be used within 30 min.** Remove and discard the Pretreat Solution. Wash the plate three times with 250 µl/well of 1x Wash Solution.
3. **Primary antibody binding.** Serially dilute the antiserums (or samples) with Dilution Solution. Add 100 µl per well and incubate at room temperature (about 25°C) for 30 minutes. Blank (without serum) and negative controls (using a negative serum) should be used as controls and for data analysis. After incubation, remove and discard the solution. Wash the plate two times with 200 µl/well of 1x Wash Solution.
4. **Incubation with ELISA Solution.** Add 100 µl/well of ELISA Solution and incubate for 20 minutes at room temperature. Then wash five times with 200 µl/well of 1X Wash Solution.
5. **Development with TMB.** Add 100 µl of One-Solution Microwell TMB substrate and incubate at room temperature for 15 minutes. Shake gently for best results.
6. **Stopping reaction.** Add 100 µl/well of Stop Solution to stop the enzyme reaction (the blue reaction mixture will turn yellow). Measure absorbance at 450nm. The color should be read within 30 minutes.
7. **Titer determination.** The antiserum titer is defined as the lowest dilution giving an absorbance three times higher than the standard deviation of the negative blank.

VII. EXAMPLES

Comparison of Express™ ELISA with classical ELISA for polyclonal antibody titer determination:

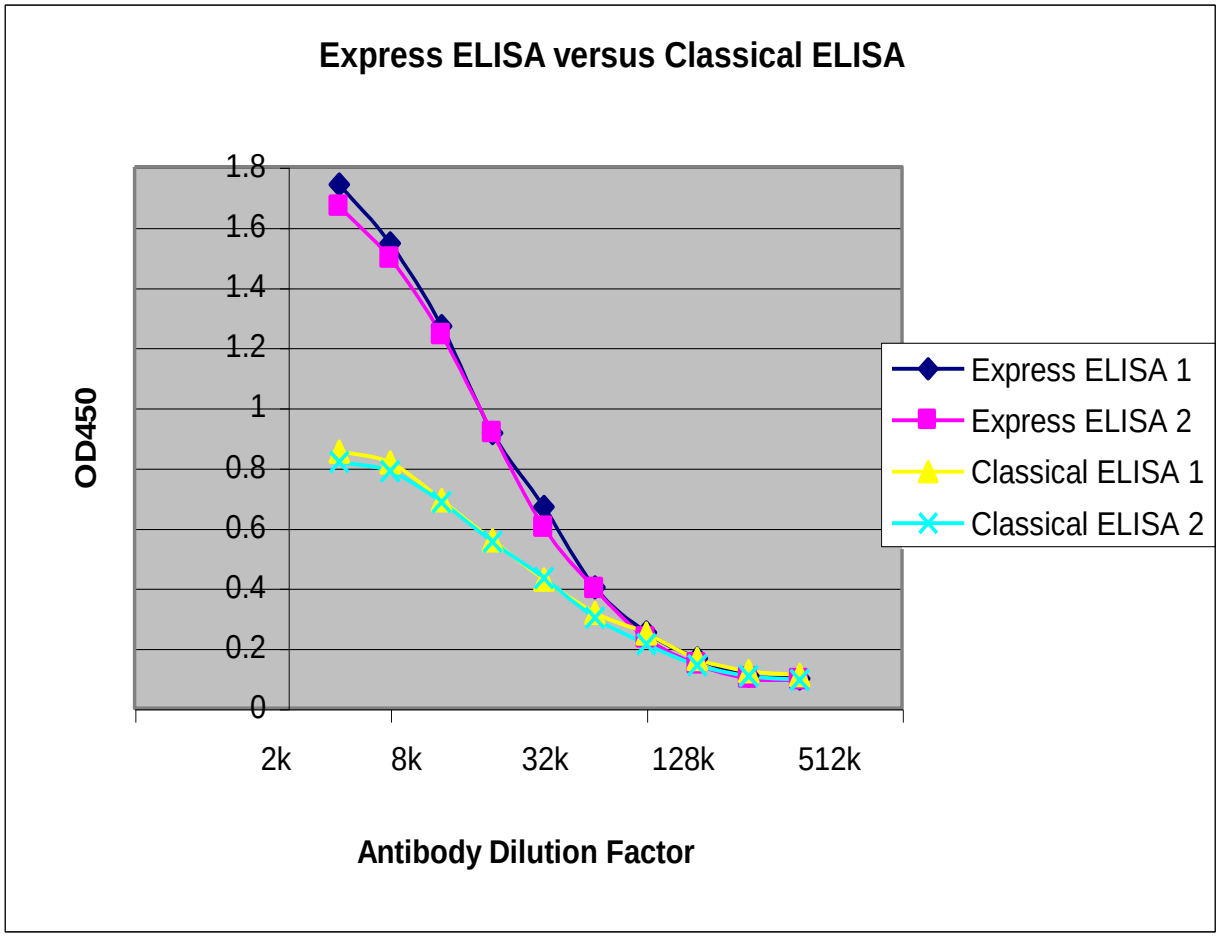
Express™ ELISA was compared with classical ELISA for the determination of rabbit antibody titer.

A and B: Express ELISA following Express™ ELISA procedure.
C and D: Classical ELISA following Classical ELISA procedure.

Detailed Procedure	Express™ ELISA (A and B)	Classical ELISA (C and D)
Coating	5 min	2 hrs
Blocking	5 min	1 hr
Primary Antibody Binding	30 min	1 hr
Secondary Antibody Binding	20 min	1 hr
Total Time	I hr	5 hrs

The antibody titer is shown in red.

Rabbit Anti-GST Antibody Titer Determination												
A、B： Express ELISA; **C、D**： Classical ELISA												
	Blank	Neg. Con.	1k	2k	4k	8k	16k	32k	64k	128k	256k	512k
A	0.052	0.064	1.742	1.546	1.27	0.915	0.67	0.402	0.253	**0.166**	0.109	0.099
B	0.05	0.057	1.668	1.495	1.242	0.916	0.601	0.397	0.233	**0.147**	0.099	0.095
C	0.048	0.076	0.854	0.817	0.693	0.558	0.429	0.318	0.249	**0.165**	0.124	0.111
D	0.044	0.059	0.819	0.789	0.685	0.554	0.434	0.301	0.214	**0.144**	0.108	0.094



Comparison of Express™ ELISA with classical ELISA:

1. Express™ ELISA is much faster than classical ELISA with regards to antibody titer determination.
2. Express™ ELISA has better linearity and a wider dynamic range.

VIII. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

Problem	Probable Cause	Solution
The OD_{450} is too low.	Too little antigen is coated on plates.	Do not dilute the antigen with any other buffer except ddH_20 and coating buffer. Use an ELISA plate (not a tissue culture plate). Use the antigen solution in Coating Buffer within 30 min.
	There is not much antibody in the serums or samples.	Improve antibody production procedure.
	The primary antibody has a low affinity for the antigen.	Improve antibody production procedure.
OD_{450} of blank or negative is too high.	Blank or negative is contaminated.	Avoid contamination of kit reagents and work at a clean place.
	There is carryover of unbound reagents, especially HRP-labeled reagent.	Make sure enough wash solution is added to each well.
	The wavelength of the reader is set at the wrong wavelength.	Check the wavelength setting.

IX. ORDERING INFORMATION

Express™ ELISA Kit: L00251 for rabbit primary antibody.
L00252 for mouse primary antibody.
L00253 for goat primary antibody.

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Patent Pending.

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