gRAD
OneDetection Kit

Revision: gRAD(1)2015-09
Please read this Instruction for Use carefully

Sections

Application
The gRAD OneDetection-kit is intended for the self development of a rapid lateral flow test. The kit is for research use only. Not for use in diagnostic procedures.

Introduction
This is the Instruction for Use for the gRAD OneDetection Kit (REF gRAD(1)-Kit). The gRAD OneDetection Kit contains components designed to easily develop custom lateral flow devices. As the gRAD OneDetection strip is generic, the use of two analyte specific antibodies: a biotinylated capture antibody and a gold colloid conjugated detection antibody that are mixed in an optimized concentration – allows both qualitative and semi-quantitative measure with the gRAD OneDetection strip. The Naked Gold Conjugation Kit allow optimized conjugation of gold colloid to the chosen detection antibody and the Sample Dilution Buffer is used to apply the specimen to the gRAD oneDetection strip. Use of the supplied calibration card allows both qualitative and semi-quantitative measure with the gRAD OneDetection strip.

Kit Content
The gRAD OneDetection -Kit (REF gRAD(1)-Kit) contains

REF	Contents	Quantity
gRAD(1)-120	gRAD OneDetection – 120 strips	6 x 20 strips
NGIB18-1	Naked Gold Conjugation Kit (20 nm and 40 nm):	1 box
	Naked Gold Sol 20 nm*	9 mL
	Naked Gold Sol 40 nm*	9 mL
	Buffer solution A – Cap with black dot	1 mL
	Buffer solution B – Cap with green dot	1 mL
	Buffer solution C – Cap with Blue dot	1 mL
	Buffer solution D – Cap with Red dot	1 mL
	Stabilizing buffer – Green cap	2 x 2 mL
SDB50	Sample Dilution Buffer	50 mL
-	Calibration Card	6 cards
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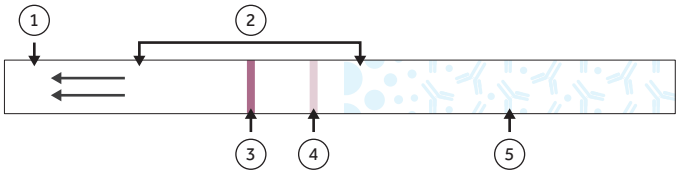
*A sol denotes a colloid solution of solid particles i.e. gold particles
The strips (gRAD(1)-120), the Gold Conjugation Kit (NGIB18-1) and the Sample Dilution Buffer (SDB50) can be purchased separately.

Reagents required but not provided
1. A biotinylated capture antibody (derived from mouse, rabbit or goat).
2. A detection antibody (derived from mouse, rabbit or goat) to be conjugated with the Naked Gold Conjugation Kit provided in the kit.
3. Samples with analyte in known concentrations.
4. Plastic tubes, pipette tips etc.

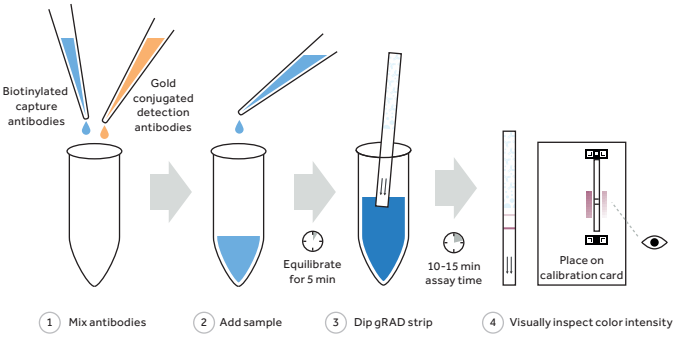
Assay principle and structure of the gRAD OneDetection strip



1. Absorption pad soaks fluid.
2. Analytical membrane transports fluid by capillarity.
3. Test line composed of biotin-binding protein captures biotinylated antibody. It becomes visible if the biotinylated antibody is in a complex with gold conjugated detection antibody and analyte.
4. Control line composed by a mix of mouse, rabbit and goat antibodies captures uncomplexed antibodies. It becomes visible if gold conjugated antibody is captured by it and thus proves that the sample has passed the test line.
5. Wicking pad drags the fluid through the analytical membrane.

How to run a developed assay based on the gRAD OneDetection system
1. The biotinylated capture antibody and the gold conjugated detection antibody are mixed.
2. The sample is added. It is recommended that the sample is incubated for 5 minutes with the biotinylated capture antibody and the gold conjugated detection antibody, but this may not be necessary for many applications. The analyte will form a complex with the capture and detection antibodies.
3. The gRAD OneDetection strip placed in the solution following the orientation given by the arrows on the strip, and left to absorb the liquid into the absorption pad. The antibodies-sample complex mixture will flow by capillary force over the test and control lines. The biotinylated capture antibody – free and in complex – will bind to the test line. Uncomplexed gold detection conjugate will bind to the control line. After an assay-specific incubation time – typically between 10 and 15 minutes – the test line will be saturated and a stable color intensity can be observed.
4. The color intensity can be estimated using the calibration card. The color intensity depends on the concentration of analyte, and thus on the ratio between the free biotinylated antibody and the sample-antibodies complex.

Figure 1 How to run a gRAD OneDetection test



Precautions
1. For Research Use only. Not for use in diagnostic procedures.
2. This kit should only be used by qualified laboratory staff.
3. All assays developed with the gRAD OneDetection Kit should be validated by the user before use.
4. Detection antibodies must be from either mouse, rabbit or goat. Otherwise they will not be captured by the control line.
5. All polyclonal antibodies must be affinity purified with the antigen of interest. The use of antisera and protein A and G purified antibodies will result in a poor test response.
6. Excess biotin from the capture antibody labelling must be removed before use. Excess biotin in the sample may interfere with the assay.
7. The test line must be inspected within 10-15 minutes. Drying and storage of the strip will affect the line intensity.
8. Do not bend the strip. The strip will not be able to transport fluid if there is not contact between absorption pad and analytical membrane.
9. Do not pre-wet the strips.
10. Avoid release into the environment. Dispose containers and unused contents in a safe way and in accordance with national and local regulations.

Stability and storage
All parts and components of the kit should be stored cooled at 2 - 8°C. Do not freeze. The expiry of each component is indicated on the box. Bring reagents to room temperature before using.

Preparation of reagents for an assay based on the gRAD OneDetection Kit
This section is meant to give an overview of the procedure – detailed procedures can be found at www.bioporto.com/gRAD-procedures.aspx.

The **capture antibody** provided by the user must be biotinylated by standard methods. Avoid free biotin in the antibody solution as it will inhibit the reaction between capture antibody and test line. An example of biotinylation protocol can be found at www.bioporto.com/gRAD-procedures.aspx.

The **detection antibody** provided by the user must be conjugated to gold particles using the Naked Gold Conjugation Kit included. Antibodies spontaneously adsorb to the surface of colloidal gold particles in buffers of similar pH to the antibody's isoelectric point. The Naked Gold Conjugation Kit includes four buffers (A, B, C and D), which allows creation of buffer solutions of a pH range of 5-11 by simply combining these buffers in different proportions. The detection antibody is added to the different buffer solutions and then added to the gold particle solution and incubated for 30 minutes. The reaction is stopped by adding the Stabilizing Buffer.

Successful conjugations are found by two simple tests. First a visual inspection – if the pH is too low the gold sol will aggregate and the color of the mixture will become darker. These mixtures cannot be used for immunoassays and should be discarded. The second test is a salt test where salt is added to the reaction mixtures before addition of stabilizing buffer. If the gold particles are not conjugated the salt will aggregate the particles and the mixture will change color. Use mixtures with no or only a slight change in color for further testing.

A detailed manual on the gold conjugation can be found at www.bioporto.com/gRAD-procedures.aspx.

Sample material should be liquid in the form of urine, serum, plasma, drinking water or solid material dissolved in the sample diluent.
Samples must be diluted in Sample Dilution Buffer. The Sample Dilution Buffer must be brought to room temperature before use.

How to develop an assay based on the gRAD OneDetection Kit
The development of a test consists of three steps:
1. Finding the analyte concentrations that give a signal (visible test line)
2. Optimization of the biotinylated capture reagent
3. Optimization of the gold conjugated detection reagent

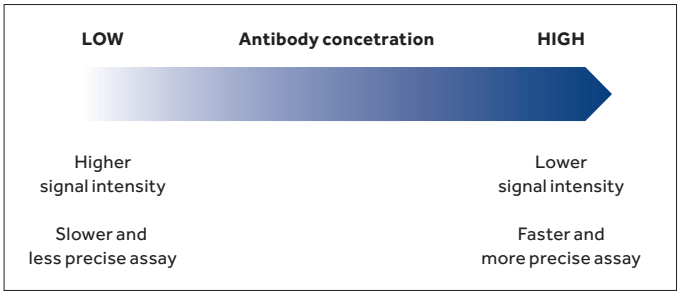
It is recommended to perform more than one optimization round.

1. Finding the analyte concentrations that give a signal
The first step is to get a signal. Choose a standard assay set-up, for example:

A. Make a reaction mix consisting of:
5 µL of 0.2 mg/mL biotinylated capture antibody solution
5 µL Gold conjugated detection antibody solution
100 µL sample solution (suggested analyte concentrations: 0.1 ng/mL to 1 µg/mL)
B. Incubate the reaction mix for 5 minutes before dipping the gRAD OneDetection strip into it
C. Wait until a visible test line appears – 10 to 15 minutes
D. Visually access the color intensity of the test line using the calibration card
E. Repeat for different analyte concentrations and build a calibration curve

2. Optimization of the biotinylated capture reagent

(1) Test different antibody conentrations – e.g. 0.5 - 20 µg/mL.
In general, it is found:



(2) It is recommended to choose a fixed assay time from assay start to reading
=> Better level of comparison of the different conditions
(3) Additonal factor which could be considered to optimize
- Amount of biotin on the capture antibody

3. Optimization of the gold conjugated detection reagent

(1) Vary the volume of gold conjugated decection antibody in the test
- The test line signal will increase when increasing the volume of detection antibody
- Inceasing the volume of detection antibody will also increase the background color of the whole strip

(2) Find the best compromise between test line signal and background on the strip

Means of preservation When the assay has been optimized it is suggested to make a large volume of the biotinylated capture antibody and gold conjugated detection antibody and aliquot them into ready-to-use volumes. These can either be stored frozen or freeze-dried. The stability must be tested by the user. To keep records of the results, it is recommended that pictures of the strips are taken during the development process, after running a test, as drying and long time storage may affect the color intensity of the test line.

Liability
This Kit is only intended for use by qualified personnel carrying out research activities. If the recipient of this test passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at recipient's own risk secure in favor of BioPorto Diagnostics A/S all limitations of liability herein.

Symbols

REF	Catalogue number		Do not reuse
LOT	Batch code		Manufacturer
	Consult instructions for use	8°C 2°C	Temperature limitation
	Use by		

Related products:

REF	Product Name	Quantity
gRAD(1)-120	gRAD OneDetection – 120 strips	6 x 20 strips
NGIB18-1	Naked Gold Conjugation Kit (20 nm and 40nm)	1 x 9 mL of each
NGIB18-2	Naked Gold Conjugation Kit (20 nm)	2 x 9 mL
NGIB18-3	Naked Gold Conjugation Kit (40 nm)	2 x 9 mL
SDB50	Sample Dilution Buffer	50 mL

ANTIBODIES
Wide range of unique monoclonal antibodies - Go to www.bioporto.com
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