TECHNICAL BULLETIN

FSL-A+B(tri) Serologic Teaching Kit

www.kodebiotech.com

Kit catalogue number		109730-2-TUO
Components in kit:		Reagent is sealed in a single tamper-proof outer.
		1 × polypropylene vial containing a mixture of 0.5 mg of FSL-A (FSL-A(GALNa3[Fa2]GALb)-SA1-L1) and 1.5 mg of FSL-B (FSL-B(GALa3[Fa2]GALb)-SA1-L1). (When reconstituted produces 2 mL of Stock Solution which can be aliquoted and stored frozen).
Storage		Store unopened kits at below 0°C, preferably at minus 10°C or below. Use frozen reconstituted Stock Solution within 12 months.
Reagents not supplied		PBS (phosphate buffered saline or a red cell preservative solution) Monoclonal ABO reagents (various) Washed hard-packed group O red blood cells
License		Teaching Use Only – not for R&D or diagnostic use
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PRODUCT DESCRIPTION

Many teaching/training laboratories do not have access to a source of specimens that will give a full range of serologic reaction grades, or those representative of ABO weak subgroups. As a consequence many laboratory staff may lack a learning opportunity or continued experience in detecting weak serologic reactions. The ability to manually score serologic reactions is important particularly as part of disaster response planning, as laboratories may need to be relocated to areas without support of automated machines. In such situations staff need to be both competent and confident in their ability to manually perform serology. Group O RBCs can be controllably modified[1] (koded) with blood group A and/or B Function-Spacer-Lipid (FSL) constructs[2] to mimic the full serological range of weak ABO subgroups[3]. These so-called kodecytes[1,4] (KODE™ construct modified cells) already have a range of uses[1-9] including quality control systems[4] and will react with most monoclonal anti-A and B blood grouping reagents. The quality (sensitivity and specificity) of various monoclonal reagents varies significantly[10].

This FSL-A+B(tri) Serologic Teaching Kit has been specifically designed for teaching purposes to create red cells (kodecytes) expressing reproducible and controlled levels of blood group A and B antigens to group O red cells[1,2]. The aim is create kodecytes that can determine and improve the skill level of the operator to detect weak serologic agglutination reactions that may be encountered when blood typing weak phenotypes of blood group A or B.

FSL-A+B(tri) Serologic Teaching Kit is a mixture of KODE™ technology FSL blood group A and B trisaccharide constructs designed for teaching/training purposes. This product contains a mixture of 0.5 mg of FSL-A(tri) and 1.5 mg of FSL-B(tri). All FSL constructs disperse in saline solutions and spontaneously and stably incorporate into cell membranes.

The features of the kit (and bulletin) include:

- Ability of users to create their own kodecytes panels
- Each 0.2 mL of Stock Solution (2.0 mL) will result in 7 tubes each containing appropriately 5 mL of a 3-5% suspension of kodecytes covering the serologic grade scoring range from 4+ to negative
- Advanced panels covering a larger serologic grade range can also be created
- Panels can be created when required and customized to user needs
- Panels can be standardized between years and institutions/organizations
- Ready-to-use worksheets are freely available

DEFINITIONS

- *FSL construct:* All KODE™ FSL constructs (Figure 1) consist of three essential designable features; a functional component (F), a spacer (S), and a diacyl lipid (L).
- *FSL-A:* The F group of FSL-A(tri) is comprised of the blood group A trisaccharide GalNAcα3(Fucα2)Galβ which is conjugated via an $O(CH_2)_3NH$ spacer (SA1) to an activated adipate derivative of dioleoyl phosphatidylethanolamine (L1).
- *FSL-B:* The same as FSL-A except the F group is comprised of the blood group B trisaccharide Galα3(Fucα2)Galβ
- *Kodecyte:* A cell modified (koded) with an FSL construct. Kodecytes are termed by the construct and the FSL concentration (in micrograms/mL) used to create them. For example kodecytes created with a 100 µg/mL solution of FSL-A would be termed A100 kodecytes. If multiple FSL constructs are used then the definition is expanded accordingly, e.g. A100+B300 kodecytes are created with a solution of 100 µg/mL solution of FSL-A and 300 µg/mL solution of FSL-B. The "+" symbol is used to separate the construct mixes, e.g. A100+B300. If FSL concentrations are constant then the µg/mL component of the terminology can be dropped, e.g. A kodecytes. Alternatively unrelated constructs such as FSL-A and FSL-biotin will create A+biotin kodecytes, etc. If different cells are used in the same study then inclusion of the cell type into the name is recommended, e.g. RBC A100 kodecytes vs WBC A100 kodecytes, or platelet A100 kodecytes, etc.
- *Koded:* A cell, virus or surface (membrane), which has a coating of FSL constructs.
- *Koding:* The process of contacting a surface/membrane with an FSL construct
- *Kodevirion:* A koded virus[12]

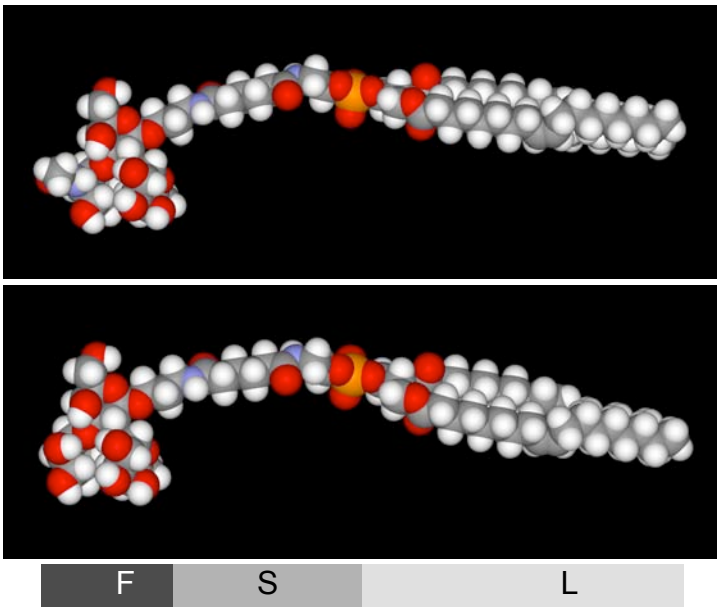
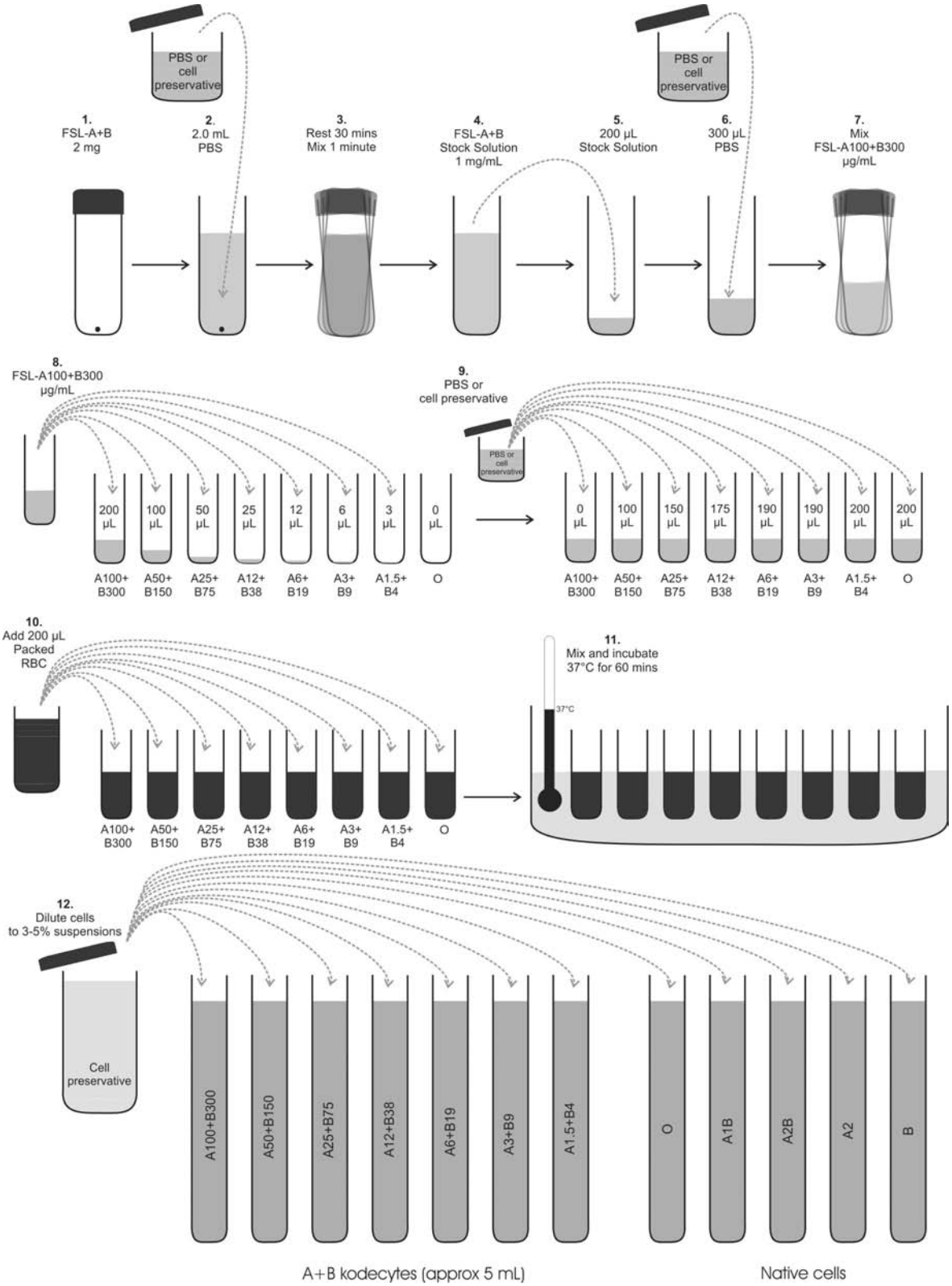


Figure 1: FSL-A (upper image) and FSL-B (lower image) constructs in one possible conformation in vacuum. The N-acetyl residue that defines the difference between these two antigens can be seen (at 9 o'clock) in FSL-A(tri).

FIGURE 2: PREPARATION OF KODECYTES (Steps relate directly to steps in the following method notes)



METHOD – PREPARATION OF KODECYTES

The following procedure is depicted graphically in Figure 2. This procedure can be done either by technicians prior to the laboratory or by users in pairs or groups of up to a maximum of 4 users. This procedure produces about 5 mL of each cell suspension and every user then requires 1.1 (min) - 1.5 (max) mL of each cell suspension for practical exercises 1-3.

Approximate time to complete procedure = 1.5 hours

NOTE: see Application Note #1 for safety issues.

1. Open the vial – note that the 2 mg of product at the bottom of the tube is probably not visible.
2. Reconstitute the 2 mg FSL-A+B(tri) Serologic Teaching Kit as a 1 mg/mL stock solution by the addition of 2.0 mL of PBS or red cell preservative solution (Application Note #2).
3. Allow to rest for 30 minutes then vortex or sonicate for 60 seconds.
4. Use the 1mg/mL stock solution within 1 week when stored at 4°C and aliquot any unused Stock Solution into vials and freeze (see Application Note #3).
5. Aliquot 200 µL of stock solution into a new clean tube (preferably with a screw cap).
6. Dilute with 300 µL of PBS or red cell preservative solution to create 500 µL of a working solution containing 100 µg/mL of FSL-A and 300 µg/mL of FSL-B.
7. Mix the FSL-A100+B300 working solution by vortex for a few seconds
8. The koding process is performed by making dilutions of the FSL-A100+B300 working solution. In the example shown (Figure 2) serial halving dilutions are prepared by direct dispensing of product. Alternatively serial two-fold dilutions can be prepared (see Application Note #4). Dispense 200, 100, 50, 25, 12, 6, or 3 µL of FSL-A100+B300 into seven tubes labeled A100+B300, A50+B150, A25+B75, A12+B38, A6+B19, A3+B9 and A1.5+B4 respectively. Also prepare an O tube into which no product is added. Tubes should be at least 5 mL capacity and have screw caps if it is intended that the suspensions will be used at a later date
9. Correct the volume of all tubes to 200 µL by the addition of PBS or red cell preservative solution. As the correction volume is non-reactive the delivery volume can be rounded to the nearest 10 µL volume.
10. Add 200 µL of washed hard-packed group O red cells (Application Note #5) to all tubes to create a 50% suspension.
11. Mix and incubate at 37°C for 60 minutes. Resuspension by gentle mixing at 30 minutes is recommended.
12. Dilute the 50% suspension with a cell preservative solution by eye to 3-5% range (see Application Note #6).
13. Prepare natural controls such as A_1B, A_2B, A_2 and B for comparative and parallel testing (see Application Note #7)
14. Label the series of kodecytes prepared with the initials of the users. This identification is required to identify issues in preparation.
15. Store the suspensions at 4°C. Kodecytes will remain stable for the normal duration of unmodified cells

Reagents & Equipment Required but Not Provided (excluding optional steps)

- Ultrasonic bath/sonicator or vortex
- 37°C water bath
- Centrifuge for preparing packed cells
- Variable dispensing pipettors
- Red cell preservative/storage solution or Phosphate buffered saline
- Permanent marker pens

PRACTICAL EXERCISE 1

Monoclonal Reagents vs Kodecytes & Native Cells

The aim of this exercise is to observe a range of serologic reaction grades and differences in sensitivity of different reagents in manual tube platforms

Users should undertake this procedure individually. Each user requires approximately 0.5 mL of each kodecyte and native cell suspension to undertake this practical exercise.

Approximate time to complete procedure = 0. 5 hours

NOTE: see Application Note #1 for safety issues.

1. Select up to 4 anti-A reagents and up to 4 anti-B reagents (see Application Notes #8 & 9). Record the details of these reagents on the worksheet (e.g. Tables 1 & 2).
2. Label a series of serologic reaction tubes for all 7 kodecyte suspensions and the native group O, A_1B, A_2B, A_2 and B cells for EACH reagent to be evaluated. Label the first tube of each

series with the ID of the reagent being evaluated.
3. Place one drop of appropriate red cells (kodecytes and native cells) into each labeled tube
4. Add one drop of monoclonal reagent to the appropriate series of red cells. It is preferable to do these one or two reagents at a time, so that incubation times between reagents and cells are similar.
5. Mix
6. Centrifuge the tubes in an immufuge or similar manual serological centrifuge (see Application Note #10)
7. Grade (Application Note # 11) and record serologic reactions (an example of typical results is shown in Table 2)

Reagents & Equipment Required but Not Provided (Additional to above)

- Serologic reaction tubes
- Monoclonal reagents
- Optical reading devices (if any)
- Transfer pipettes

SUGGESTED WORKSHEET LAYOUT FOR PRACTICAL EXERCISE 1

	A+B kodecytes							Native cells				
Reagent ID	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

Table 1: Suggested worksheet layout for Practical Exercise 1. This worksheet is for the user to record the results they obtain from testing various undiluted monoclonal reagents against kodecytes and native cells (e.g. Table 2). See Appendix for full versions of worksheets (Appendix 16).

EXAMPLE RESULTS FOR PRACTICAL EXERCISE 1

	A+B kodecytes							Native cells				
Reagent ID	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
#1	4+	3+	2+	2+	1+	-	-	-	4+	3+	4+	-
#2	4+	3+	2+	1+	-	-	-	-	3+	4+	-	4+

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date
#1	Anti-A	GoodClone, KBML	ABC12345-01	June 2014
#2	Anti-B	GoodClone, KBML	BCD12345-02	January 2013

Table 2: An example of typical results for Practical Exercise 1 for two reagents. Results will vary depending on sensitivity and specificity of reagent used[11] and also see Application Note # 9.

PRACTICAL EXERCISE 2

Diluted Reagent vs Kodecytes & Native Cells

The aim of this exercise is to observe consequences of reagent deterioration (in this case caused by dilution) on ability of native cells and kodecytes to react. A large range of serologic grades will be observed in this chequerboard titration (antigen and antibody dilutions).

Users should undertake this procedure individually. Each user requires 0.5 mL of each kodecytes suspension and native cells to undertake this practical exercise.
Approximate time to complete procedure = 0.7 hours

NOTE: see Application Note #1 for safety issues.

1. Select one potent reagent from the previous exercise. Record the details of this reagent on the worksheet (e.g Tables 3 & 4).
2. Prepare 1 mL serial two-fold dilutions in PBS from 1:2 to 1:64 of this reagent (Application Note #12)
3. Label a series of serologic reaction tubes for all 7 kodecyte suspensions and the native group O, A_1B, A_2B, A_2 and B cells for EACH of the 6 reagent dilutions to be evaluated. Label the first tube of each series with the ID and dilution of the reagent being evaluated.
4. Place one drop of appropriate red cells (kodecytes and native cells) into each labeled tube
5. Add one drop of reagent dilution into the appropriate series of red cells. It is preferable to do these one or two reagents at a time, so that incubation times between reagents and cells are similar.
6. Mix
7. Centrifuge the tubes in an immufuge or similar manual serological centrifuge (see Application Note #10)
8. Grade and record serologic reactions (an example of typical results is shown in Table 4)

SUGGESTED WORKSHEET LAYOUT FOR PRACTICAL EXERCISE 2

Reagent Dilution	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
1:2												
1:4												
1:8												
1:16												
1:32												
1:64												

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

Table 3: Suggested worksheet layout for Practical Exercise 2. This worksheet is for the user to record the results they obtain from testing serial dilutions of a monoclonal reagent against kodecytes and native cells (e.g. Table 4). See Appendix for full versions of worksheets (also see Application Note #16).

EXAMPLE RESULTS FOR PRACTICAL EXERCISE 2

Reagent Dilution	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
1:2	4+	4+	3+	2+	1+	-	-	-	4+	4+	4+	-
1:4	4+	4+	3+	2+	1+	-	-	-	4+	4+	4+	-
1:8	3+	3+	3+	1+	-	-	-	-	4+	4+	4+	-
1:16	3+	3+	2+	1+	-	-	-	-	4+	4+	4+	-
1:32	2+	2+	2+	1+	-	-	-	-	4+	4+	4+	-
1:64	2+	2+	1+	-	-	-	-	-	4+	3+	4+	-

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date
#1	Anti-A	GoodClone, KBML	ABC12345-01	June 2014

Table 4: An example of typical expected results for Practical Exercise 2. Results will vary depending on sensitivity and specificity of reagent used (see Application Note # 9).

PRACTICAL EXERCISE 3

Blind Testing

The scores obtained in a known dilution format can be anticipated. The aim of this exercise is to grade a range of unknown and unpredictable serologic reaction grades.

Users should undertake this procedure individually but will need to match up with a partner to exchange materials. Each user requires 0.5 mL of each kodecytes and native cell suspension to undertake this practical exercise.
Approximate time to complete procedure = 1.0 hours

NOTE: see Application Note #1 for safety issues.

1. Select your panel of kodecytes and natural cells and label a series of tubes 1 – 12 to represent each tube
2. On the worksheet (e.g. Tables 5 & 9) randomly assign a code number between 1 and 12 to each cell suspension. This process will “blind” the identity of the samples
3. Transfer 250µL of the contents from the kodecytes or native cells tube into your randomly assigned and matched tube.
4. Place the panel in numerical order from 1 to 12
5. Select up to 2 reagents you would like this tested against this blinded panel.
6. Now exchange your randomly assorted blinded panel (but which you have the code for) together with the two reagents to another user. Do not reveal your random code yet.
7. You should now also have received a blinded panel of cells from another user, together with two monoclonal reagents. Record the details of these reagents on the worksheet (e.g. Tables 6 & 8).
8. Label a series of serologic reaction tubes for all 12 cell suspensions for EACH of the two reagents you have. Label the first tube of each series with the ID of the reagent being evaluated.
9. Place one drop of appropriate red cells (labeled 1 - 12) into each labeled tube
10. Add one drop of monoclonal reagent to the appropriate series of red cells.
11. Mix
12. Centrifuge the tubes in an immufuge or similar manual serological centrifuge (see Application Note #10)
13. Grade and record serologic reactions (e.g. Table 8)
14. Exchange information with the person who gave you the “blinded” panel. Record the anti-A and anti-B results expected (those obtained by the other user) and then mark your own results (e.g. Tables 8 and 10).
15. Retest any major discrepancies (see application note #13)

SUGGESTED WORKSHEET LAYOUT FOR PRACTICAL EXERCISE 3

	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
Code												
Expected A												
Expected B												

Table 5: Suggested worksheet layout for Practical Exercise 3. This worksheet is for the user to record their randomized 1-12 code (step 2) that they assign to the cell suspensions (e.g. Table 8) and their expected score. They will exchange this panel with another user but give them these details only after they have obtained their own results.

	Blinded randomized cells (kodecytes and native cells)											
	1	2	3	4	5	6	7	8	9	10	11	12
Result A												
Result B												

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

Table 6: Suggested worksheet layout for Practical Exercise 3. This worksheet is for the user to record the results they obtain from testing the blinded panel given to them by another user (only identified by a number 1-12) (e.g. Table 9).

	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
Code*												
Result A												
Expected A												
Result B												
Expected B												
Retest #1												
Retest #2												

* the code and expected results are provided once you have obtained results for the blinded panel.

Table 7: Suggested worksheet layout for decoding Practical Exercise 3. This worksheet is for the user to record the code assigned to the cells suspensions (and the expected score) by another user. This information should not be made available until table 6 has been completed. Transcribe results obtained to this sheet. Any results that vary significantly from the expected should be retested (Application Note # 13). See Appendix for full versions of these worksheets (also see Application Note #16).

EXAMPLE RESULTS FOR PRACTICAL EXERCISE 3

	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
Code	***3***	***1***	***2***	***9***	***6***	***5***	***11***	***7***	***4***	***8***	***12***	***10***
Expected A	*4+*	*3+*	*2+*	*2+*	*1+*	-	-	-	*4+*	*3+*	*4+*	-
Expected B	*4+*	*3+*	*2+*	*1+*	-	-	-	-	*3+*	*4+*	-	*4+*

Table 8: An example of typical coded results for Practical Exercise 3. The information in this worksheet was prepared by the first user and exchanged with another user (after they have obtained their results).

	Blinded randomized cells (kodecytes and native cells)											
Reagent ID	1	2	3	4	5	6	7	8	9	10	11	12
Result A	-	4+	-	4+	3+	-	1+	3+	-	1+	1+	4+
Result B	-	3+	4+	4+	3+	-	1+	3+	-	2+	-	1+

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date
#1	Anti-A	GoodClone, KBML	ABC12345-01	June 2014
#2	Anti-B	GoodClone, KBML	BCD12345-02	January 2013

Table 9: An example of typical results for Practical Exercise 3. At this stage it is unknown what the results should be or against which cells they belong to.

	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
Code*	***4***	***5***	***10***	***7***	***11***	***1***	***6***	***9***	***2***	***8***	***12***	***3***
Result A	4+	3+	1+	1+	1+	-	-	-	4+	3+	4+	-
Expected A*	*4+*	*3+*	*2+*	*2+*	*1+*	-	-	-	*4+*	*3+*	*4+*	-
Result B	4+	3+	2+	1+	-	-	-	-	3+	3+	1+	4+
Expected B*	*4+*	*3+*	*2+*	*1+*	-	-	-	-	*3+*	*4+*	-	*4+*
Retest #1												
Retest #2											-	

* the code and expected results are provided once you have obtained results for the blinded panel.

Table 10: An example of typical results analysis for Practical Exercise 3. After obtaining results (table 9) the user should now obtain the code from the person who prepared the panel (Table 8) and record these into the worksheet (table 10) the results expected for each cell suspension (grey squares). The user should now transcribe their results obtained onto this worksheet (white boxes) and analyse for variances. In the example given with one exception the results did not vary significantly (Application Note # 13) from the expected. The one exception was a 1+ positive result was obtained against anti-B reagent #2 for suspension 12 (the A_2 cell). On retesting this was found to be negative – the correct result.

PRACTICAL EXERCISE 4

Expanded Panel and Blind Testing

It is generally observed that grades go from 4+ to 1+ over 4 tubes in a serial two-fold dilution. Where these four tubes occur depends on the sensitivity and specificity of the reagent being used (see Application Note 10). The range of strong positive to weak positive can be expanded by creating kodecytes each decreasing by 10% in koding levels. The aim of this exercise is improve scoring by using an expanded range of kodecytes with a large range in serologic grades when tested against a selected reagent(s). In this procedure the kodecytes are usually prepared in advance and in bulk. Selected samples (usually 6 on each occasion) are given to users over a period of up to 6 weeks (depending of volumes made).

METHOD – PREPARATION OF KODECYTES

Part of the following procedure for the preparation of an extended panel is depicted graphically in Figure 3 replaces steps 8 & 9 in Figure 2.

Each 200 µL of Stock Solution will result in 4 mL of a 3-5% cell suspension and each mL should be sufficient for 20 assays. Scale quantities as required.

Approximate time to complete procedure = 1.5 hours
NOTE: see Application Note #1 for safety issues.

1. Only one reagent is used for this exercise so select a reagent that you have sufficient of to last the duration (weeks) of this exercise. This reagent must clearly show strong positive to negative serologic grades over 3-4 tubes in a serial two-fold koding dilution. For example either of the reagents shown in Table 2 are suitable (but for this description reagent #1 will be used as the example)
2. Determine the koding concentration of the kodecyte to give the first 2+ reaction (with respect to the example in Table 2 this would be A25+B75 for reagent #1). This dilution will be used as the 50% point in the expanded panel.
3. Working backwards from this concentration select the koding concentration 2-times more concentrated (i.e. 1-tube to the left). Again with respect to Table 2 this would equate with A50+B150 for reagent #1. This is the starting 100% concentration.
4. Using the 1mg/mL Stock Solution prepare 1.2 mL of the starting 100% concentration (see Application Note #14 for volumes). Label this tube FSL-100% and/or with its actual FSL concentration (e.g. FSL-A50B150).
5. Mix the 100% working solution by vortex for a few seconds
6. The koding process is performed by making dilutions of the FSL-100% working solution. In the example shown (Figure 3) serial 10% reducing dilutions are prepared by direct dispensing of product. Dispense 200, 180, 160, 140, 120, 100, 80, 60, 40 and 20 µL of FSL-100% into 10 tubes labeled 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, and 10% respectively (or by actual concentration - see Application Note #15). Also prepare an O (0%) tube into which no product is added. Tubes should be at least 5 mL capacity and have screw caps if it is intended that the suspensions will be used at a later date.
7. Correct the volume of all tubes to 200 µL by the addition of PBS or red cell preservative solution.
8. Add 200 µL of washed hard-packed group O red cells (Application Note #5) to all tubes to create a 50% suspension.
9. Mix and incubate at 37°C for 60 minutes. Resuspension by gentle mixing at 30 minutes is recommended.
10. Dilute the 50% suspension with a cell preservative solution (see Application Note #6) by eye to 3-5%.
11. Prepare native controls such as A_1B, A_2B, A_2 and B for comparative and parallel testing (see Application Note #7)
12. Store the suspensions at 4°C. Kodecytes will remain stable for the normal duration of unmodified cells

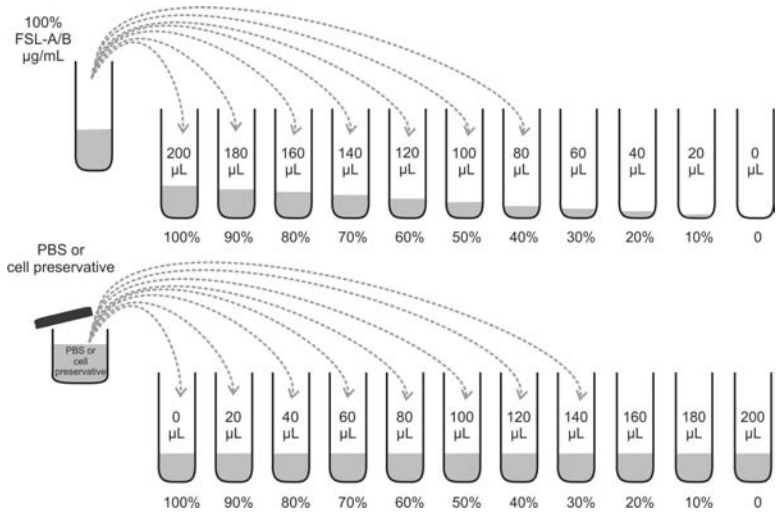


Figure 3 – Preparation of expanded kodecyte panel (FSL- dilution step)

PRACTICAL EXERCISE

Standard Monoclonal Reagent vs Expanded Kodecyte Panel & Native Cells

NOTE: see Application Note #1 for safety issues.

The aim of this exercise is to improve serologic grading competence by observing an unknown selection of cells from an expanded panel with large range of serologic reaction grades. This procedure is usually conducted over several weeks (depending of numbers of users and volumes of reagents). This procedure may fit as an opening exercise to practical laboratories or may be done on multiple occasions over a single day.

There are available 10 kodecyte preparations and a range of native cells. There is only one monoclonal reagent available. For each exercise the lecturer should select a "random" combination from the available cells. We recommend selecting only 6 suspensions for each exercise. Because weak serologic grades are to some extent influenced by cell concentrations we recommend that the cell suspensions be pre-dispensed into test tubes ready for the user analysis.

Users should undertake this procedure individually and an experienced serologist should conduct the procedure in parallel (expected results).

Preparation

1. Select 6 suspensions from the kodecytes and native cells. It is recommended that the number of true-negative reactions (i.e. not kodecytes) should be varying between 1-5 (of the 6 suspensions selected) between exercises to ensure users maintain competence in scoring negative reactions.
2. Prepare tubes labeled 1-6 for each user
3. Record the order "random" in which you want the samples to be dispensed (e.g. Tables 11 & 13)
4. Dispense 1 drop (or preferably 40-50 µL = 1 drop) of well-mixed cell suspension to each appropriate tube.
5. Make available to users the monoclonal reagent and the dispensed cell suspensions.

User Practical Exercise

Approximate time to complete each procedure = 0.3 hours

1. Add one drop of monoclonal reagent to the series of red cells.
2. Mix
3. Centrifuge the tubes in an immufuge or similar manual serological centrifuge (see Application Note #10)
4. Grade and record serologic reactions (Table 12)
5. Compare results with expected results and/or user consensus (e.g. Tables 13 & 14)
6. Where required retest (Application Note #13)

SUGGESTED WORKSHEET LAYOUT FOR PRACTICAL EXERCISE 4

	Expanded Kodecyte Panel and Native Cells														
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0	A_1B	A_2B	A_2	B
Tube ID															
Expected															

Table 11: Suggested worksheet layout for Practical Exercise 4. This worksheet is for use by the lecturer to record their randomized 1-6 code that they assign to the cell suspensions given to the user and the expected result (Table 13). The extended version of this worksheet (appendix) can also be used for data analysis. Simply record the tube number against the box identifying the cell suspension. If the same cell suspension is used twice, or more, record multiple numbers in the available box (e.g. Table 13 , the O cell was used as sample 2 and 6).

Expanded Kodecyte Panel and Native Cells						
	1	2	3	4	5	6
Results						
Expected						
Retest						

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

Table 12: Suggested worksheet layout for Practical Exercise 4. This worksheet is for the user to record the results they obtain from testing the blinded panel given to them, then the expected result and retest results (if required). See Appendix for full versions of these worksheets (see also Application Note #16).

	Expanded Kodecyte Panel and Native Cells														
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0	A_1B	A_2B	A_2	B
Tube ID				5				1	4		2,6				3
Expected				3+				1+	1+/-		-				-

Table 13: An example of typical expected results for Practical Exercise 4. The numbers of the six tubes given are recorded in this table by the lecturer together with the expected result. An extended version of this table in the appendix can be used to acquire this data for analysis.

Expanded Kodecyte Panel and Native Cells						
	1	2	3	4	5	6
Results	2+	-	-	-	1+	-
Expected	1+	-	-	1+/-	3+	-
Retest					2+	

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date
#1	Anti-A	GoodClone, KBML	ABC12345-01	June 2014

Table 14: An example of typical expected results for Practical Exercise 4. Following the exercise the user was given the expected results (table 12) and could compare their results and retest any major discrepancies (Application Note # 13). The one exception was a 1+ positive result obtained against suspension 5 (the 70% kodecytes). On retesting this was found to be a 2+ grade – within range of an acceptable result. In the upper grey area of this sheet (above the ID number) the user may optionally record the actual identity of the cells when made available.

ANALYSIS TOOLS

Assessing Performance

Because the practical exercises can be standardized analysis of results against expected outcomes can be undertaken (Application Note #17). By recording variances (Application Note #13) a pattern of capability and improvement can be established for individual users. Typically three groups of users are identified: (1) competent and confident and not requiring intervention, (2) competent but not confident and requiring further practice for confidence and (3) requiring further training. The latter group typically can be divided into 4 types (a) over-readers (consistently reading more than one grade higher than the expected result, or reading true negatives as positives), (b) under-readers (consistently reading more than one grade lower than the expected result) (c) random (unpredictable over and under reading) and (d) drifters (during the course of training some users begin to become less confident and can temporarily become either 2(a) or 2(b)). Regularly feedback and practice generally corrects all deficiencies.

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KODE™ constructs for research purposes can be obtained from Sigma-Aldrich (www.sigma.com/kode) or from KODE Biotech Materials Limited (www.kodebiotech.com).

APPLICATION NOTES

Note #1. ***Practice appropriate blood handling precautions.*** FSL-A and FSL-B constructs present no known biological or chemical risk. MSDS sheets for FSL-A and FSL-B are available from both KODE Biotech (www.kodebiotech.com) and Sigma-Aldrich (www.sigma.com/kode).

Note #2. Stock Solutions can be diluted in buffers containing protein. Stock solutions should not be diluted in buffers containing lipids (e.g. serum) or other hydrophobic products as the FSL will associate with this material and insertion into cells will be reduced. Non-LISS red cell storage/preservative solutions are preferred. Product is unstable if reconstituted in water.

Note #3. Store unopened product at below minus 10°C. Store 1 mg/mL Stock Solution aliquots at below minus 10°C and avoid repeated freezing and thawing of solutions. Frozen Stock Solution should be used within 12 months.

Note #4. Serial two-fold dilutions can be used as an alternative technique to direct dilution. Prepare labeled tubes and add 200 µL of PBS or red cell preservative solution into all tubes except the A100+B300 tube. Add 200 µL of FSL A100+B300 to both the A100+B300 tube and A50+B150 tubes. Mix the contents of the A50+B150 tube and using the "to-contain" mode transfer 200 µL to the next tube (A25+B75) and mix. Still with the same pipette continue the process until all dilutions have been made. Discard 200µL from the last dilution (so that its total volume is only 200 µL).

Note #5. Washed hard packed red cells. It is important that the red cells used are washed free of serum/plasma. Wash cells 3 times with PBS then once with red cell preservative solution. Hard pack – although this is not critical to the koding process it is to get reproducible results on future occasions or for comparison between different institutions.

Note #6. If it is intended that the kodecytes are to be kept for longer than a day then a cell preservative solution (see also Note #2) should be used at steps 2, 6, 9 and 12. However, if the cells are to be consumed within 6 hours then PBS is suitable. It is not necessary to wash the kodecytes as almost all the FSL constructs will have inserted into the cells. However, if PBS was used at an earlier stage and it is intended that the cells be stored in a cell preservative solution then one wash in cell preservative solution before preparing cell suspensions is recommended.

Note #7. Excluding weak subgroups such as A_x which clearly have weak reaction grades the relatively common blood group A_1B and A_2B red cells are the next best choice for weak expression of A and B antigens. Blood group A_1B will have reduced levels of B antigen while A_2B will have reduced levels of A antigen. Blood group O, A_2 and B are included as normal examples of absent and strong antigen expression. The O cells used should be the same cells as were used to prepare the kodecytes. A good alternative red cell expressing weak A or B antigens are cord cells, but these are not usually readily available nor is their infectious status usually known.

Note #8. Polyclonal reagents may be used, but they may show unexpected reactivity. It is established that trisaccharide A and B antigens will react with other non-ABO antibodies present in human serum[13]. Monoclonal anti-AB reagents may also be used but may show variable results depending on the nature of their formulation and blending.

Note #9. It has been recently found that although almost all MAbs will react with the trisaccharide A and B constructs a few (<5%) will have a more restricted specificity and show low affinity for trisaccharides, but good affinity for tetrasaccharides (e.g. A type 2). This should not be viewed as a performance issue of the reagent being tested, rather an indicator of specificity and affinity for trisaccharides. In general the specificity and sensitivity of the monoclonal reagent used will determine its reactivity with kodecytes. However there is considerable differences in sensitivity and specificity of commercially available monoclonal antibodies[11]. Testing in gel cards may have a more abrupt end-point than manual tube serology and may product mixed-field reactions. As with manual reactions the type and quality of reagents in the gel cards will influence the results obtained. Weak ABO serologic reactions are very sensitive to temperature, to get consistent result room-temperature should be maintained at a standard. It has been noted that a change as small as a few degrees C can alter serologic grades.

Note #10. An alternative to centrifugation is to leave the reactions to sediment for 45-60 minutes and then read the reactions directly.

Note #11. Grading serologic reactions. The reaction grading system used here is 4+, 3+, 2+ 1+ and 0/- scale.

A 4+ reaction is where cell button remains in one large clump or dislodges into a few large clumps which are macroscopically visible. A 3+ reaction is where cell button dislodges into numerous medium to large clumps which are macroscopically visible. A 2+ reaction is

where cell button dislodges into many small clumps macroscopically visible. A 1+ reaction is where cell button dislodges into very small but confirmed as definite by magnification. A 0 or – is a negative reaction where cell button dislodges and/or breaks up easily as free cells. A mf grade is a mixed field reaction where a population of positive clumps are seen in a field of a negative reaction (or as two populations in a gel card technology).

Note #12. To prepare 1 mL serial two-fold dilutions of a reagent first label a series of tubes 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. Add 1 mL of PBS to each tube. Using the "to-contain" mode add 1 mL of undiluted reagent to the first tube (1:2) and with the same pipette tip in place mix the contents of the tube. Still with the same pipette and using again using the "to-contain" mode aspirate 1 mL from the 1:2 tube and transfer to the contents to the 1:4 tube, mix. Continue the process until all dilutions have been made.

Note # 13. A major variance in score grade from the expected result is one which is >1 score grades away from the expected result, or is positive with a true negative. For example if the expected score was 2+ then scoring either a 1+ or a 3+ is considered acceptable, however a 4+ or negative result is not. Equally if a true negative (i.e. a group O cell tested against either anti-A or anti-B) scores a positive of any score then the result is a fail. If an FSL dilution series scores more than two consecutive 1+ reactions (e.g. 3+, 2+, 1+, 1+, 1+, -) then the kodecyte (shaded) scoring the 3rd 1+ reaction is also probably a fail – but may be dependent on the reagent used. An experienced serologist will be required to define and arbitrate when a reaction is considered negative, versus a grainy or sticky reaction. More advanced and skilled serologists can apply tighter criteria.

Note #14. Use the following guide to prepare 1.2 mL of FSL solutions from the 1mg/mL stock solution

	FSL Stock 1mg/mL		PBS
A100+B300	480 µL	+	720 µL
A50+B150	240 µL	+	960 µL
A25+B75	120 µL	+	1080 µL
A12.5+B37.5	60 µL	+	1140 µL

Note #15. Equivalent FSL concentrations of the kodecytes prepared by the 10% dilution method of the 100% kodecytes (which equate with those prepared by doubling dilutions).

	µg/mL		µg/mL		µg/mL		µg/mL	
	A	B	A	B	A	B	A	B
100%	100	300	50	150	25	75	13	38
90%	90	270	45	135	23	68	11	34
80%	80	240	40	120	20	60	10	30
70%	70	210	35	105	18	53	9	26
60%	60	180	30	90	15	45	8	23
50%	50	150	25	75	13	38	6	19
40%	40	120	20	60	10	30	5	15
30%	30	90	15	45	8	23	4	11
20%	20	60	10	30	5	15	3	8
10%	10	30	5	15	3	8	1	4

Note #16. Copyright-free full ready to use (or modify) versions of worksheets may also be obtained in Microsoft Word format from the authors – kiwi@aut.ac.nz or may be available on the KODE Biotech Website

Note #17. Copyright-free full ready to use (or modify) versions of analysis worksheets may also be obtained in Excel format from the authors – kiwi@aut.ac.nz or may be available on the KODE Biotech Website

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OTHER RESOURCES

ko•de•cyte |kəʊdəsʌɪt| www.kodecyte.com

KODE Biotech www.kodebiotech.com - TECHNOLOGY

YouTube – search "kodecyte"

Wikipedia – search "kodecyte" and "kodevirion"

http://www.jove.com/details.php?id=3289

DOCUMENT CONTROL

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Website: www.kodebiotech.com

Version: 20111128

Release date: 28 November 2011

WORKSHEET FOR PRACTICAL EXERCISE 1

USER NAME _____

KODECYTE PANEL ID _____

SEROLOGIC RESULTS

Reagent ID	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

WORKSHEET FOR PRACTICAL EXERCISE 2

USER NAME _____

KODECYTE PANEL ID _____

SEROLOGIC RESULTS

Reagent Dilution	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
1:2												
1:4												
1:8												
1:16												
1:32												
1:64												

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

WORKSHEET FOR PRACTICAL EXERCISE 3

USER NAME _____

KODECYTE PANEL ID _____

PANEL RECEIVER _____

CODE FOR BLINDED PANEL

	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
Code												
Expected A												
Expected B												

DO NOT DISCLOSE THIS INFORMATION UNTIL THE PANEL

HAS BEEN TESTED BY THE RECEIVER

WORKSHEET FOR PRACTICAL EXERCISE 3

USER NAME _____

KODECYTE PANEL ID _____

PANEL RECEIVED FROM _____

SEROLOGIC RESULTS

Reagent ID	Blinded randomized cells (kodecytes and native cells)											
	1	2	3	4	5	6	7	8	9	10	11	12
Result A												
Result B												

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date

ANALYSIS (TRANSCRIBE RESULTS FROM ABOVE INTO WORKSHEET FOR ANALYSIS)

	A+B kodecytes							Native cells				
	A100+B300	A50+B150	A25+B75	A12+B38	A6+B19	A3+B9	A1.5+B4	O	A_1B	A_2B	A_2	B
Code*												
Result A												
Expected A*												
Result B												
Expected B*												
Retest #1												
Retest #2												

* the code and expected results (grey boxes) are provided once you have obtained results for the blinded panel.

WORKSHEET FOR PRACTICAL EXERCISE 4

LECTURERS NAME _____ CLASS _____

KODECYTE PANEL ID _____ DATE OF TESTING _____

KODECYTE 50% FSL CONCENTRATION A_____B_____

	Expanded Kodecyte Panel and Native Cells														
	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0	A_1B	A_2B	A_2	B
Tube ID															
Expected															
Users															

WORKSHEET FOR PRACTICAL EXERCISE 4

USER NAME _____ CLASS _____

KODECYTE PANEL ID _____ DATE OF TESTING _____

Expanded Kodecyte Panel and Native Cells						
	1	2	3	4	5	6
Results						
Expected						
Retest						

Reagent ID	Specificity	Brand & Manufacturer	Batch number	Expiry date