



Kode Materials
biosurface innovation supplies



AUT Centre for
Kode Technology Innovation

TECHNICAL BULLETIN:

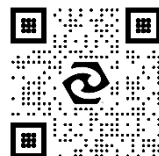
Kode Technology SARS-CoV-2 kodecytes and Function Spacer Lipid constructs.

Williams E,¹ Trent C,¹ Bovin N,^{1,2} Nagappan R,¹ Henry S¹.

1. Product description
2. Methods of SARS-CoV-2 kodecytes – preparation & serological use
3. References and further information
4. MSDS

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TECHNICAL BULLETIN:

Kode Technology SARS-CoV-2 kodecytes and Function Spacer Lipid constructs.

| | | |
|-------------------------|--|--|
| FSL construct full name | FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1 | #572257 |
| | FSL-CoV12-1255(cKFDEDDSEPVLKGVK)C-SC2-L1 | #736126 |
| Product Catalogue # | 572257-0.2-R&D or 572257-0.2-LA | (R&D = Research use only) |
| | 736126-0.2-R&D or 736126-0.2-LA | (LA = Commercial license) |
| FSL short name | FSL-CoV12-1147 | |
| | FSL-CoV12-1255 | |
| Reagents: |  | 1 × polypropylene vial containing 0.2 mg FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1. |
| |  | 1 × polypropylene vial containing 0.2 mg FSL-CoV12-1255(cKFDEDDSEPVLKGVK)C-SC2-L1. |
| Storage | Store unopened vials at below 0°C, preferably at minus 10°C or below. Use frozen reconstituted Stock Solution within 12 months. | |
| Reagents not supplied | Red cell preservative solution | |
| | Washed hard-packed group O red blood cells | |
| Disclaimer | This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices. | |
| | Purchaser must determine the suitability of the product(s) for their particular use. | |
| License | R&D – no license is required for non-commercial use. | |
| | LA – Commercial manufacture and supply requires a license from Kode Biotech Ltd. | |
| | For at least 5 years, the licensor (Kode Biotech Limited) pledges not to enforce its intellectual property rights against users of Kode™ Constructs in SARS-CoV-2 diagnostic platforms, subject to the constructs used being sourced from an authorized Kode construct supplier. In these circumstances, users of Kode™ Constructs for the development, manufacture and supply of SARS-CoV-2 diagnostic platforms will not be required to pay a royalty to Kode Biotech Limited. | |
| Publications | Nagappan R, Flegel WA, Srivastava K, Williams EC, Ryzhov I, Tuzikov A, et al. COVID-19 antibody screening with SARS-CoV-2 red cell kodecytes using routine serologic diagnostic platforms. Transfusion 2021. DOI: 10.1111/trf.16327 (1) | |

1. PRODUCT DESCRIPTION

Kode Technology utilizes a lipid fragment to attach an antigen structure onto red cells (kodecytes). Function-Spacer-Lipid (FSL) constructs for use in non-covalent cell-surface modification/engineering of cellular membranes, viral particles, liposomes, or other surfaces is well-established (2-8). The semi-rigid Spacer in this molecule is constructed via modified hexapeptide unit (Gly-Gly-Ide)₂ coupling to both amino groups of ethylenediamine and has been designed to ensure accessibility for target binding/external interactions and proper presentation of functional peptides at a cell surface as well as imparting good solubility to the construct. Electrostatic repulsion forces of the spacer's anionic groups probably favor uniform distribution of the incorporated constructs on the membrane surface (9). The diacyl phospholipid derived from unsaturated fatty acids is a prerequisite for spontaneous incorporation into cell membranes.

The protocol described here is NOT optimized for using FSL-CoV12-1147 and FSL-CoV12-1255 to make SARS-CoV-2-kodecytes for the serological detection of antibodies.

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-CoV12-1147*: The F group of FSL-CoV12-1147 is comprised of the peptide cSFKEELDKYFKNHTS, which is conjugated via a carboxymethylglycine CMG(2) (SC2) to an activated adipate derivative of dioleoyl phosphatidylethanolamine (L1).
- *FSL-CoV12-1255*: The F group of FSL-CoV12-1255 is comprised of the peptide cKFDEDDSEPVKGVK, which is conjugated via a carboxymethylglycine CMG(2) (SC2) to an activated adipate derivative of dioleoyl phosphatidylethanolamine (L1).
- *Kodecyte*: Terminology and methodology for describing FSL constructs and the resultant kodecytes is described in detail elsewhere (5). Essentially the kodecyte is described by the ID of the FSL's functional head and the micromolar (μmol/L) concentration of the FSL solution used to make it, e.g. an 1147-3-kodecyte is kodecyte made with peptide 1147 at an FSL concentration of 3 μmol/L, while an 1147+1255-1.5+2.5-kodecyte is a dual epitope bearing kodecyte made with a blend of FSL-1147 and FSL-1255 at respective concentrations of 1.5 and 2.5 μmol/L.
- *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
- *Kodeviroin*: A koded virus

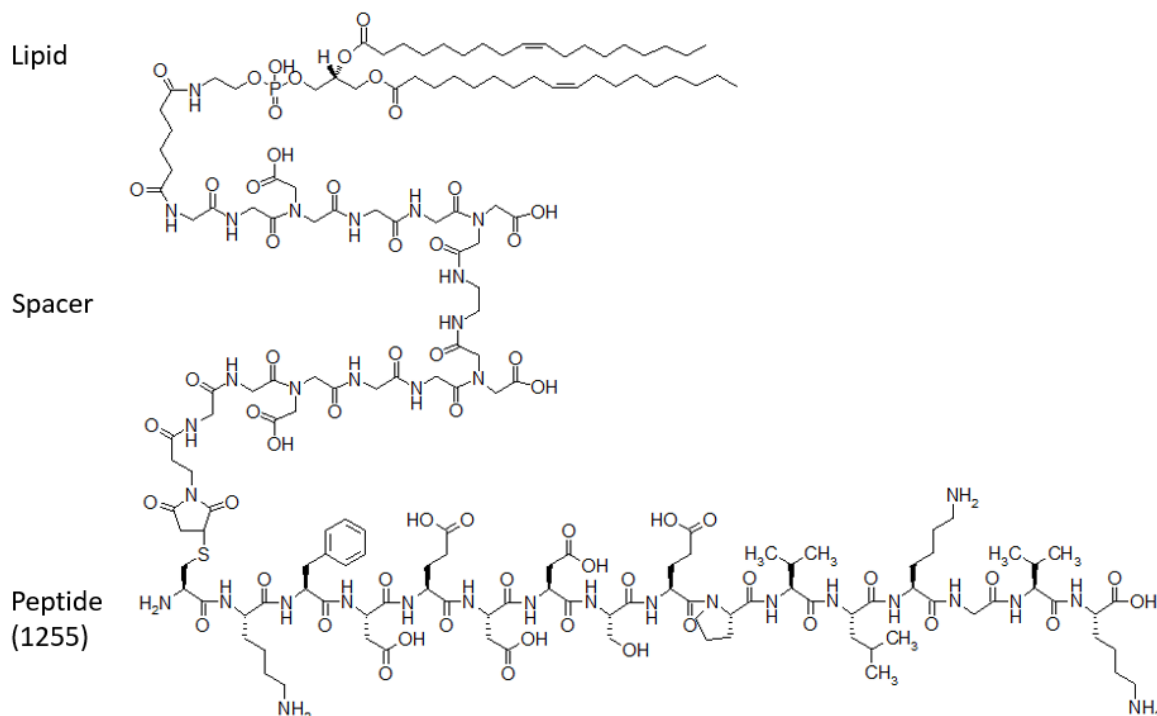


Figure 1: Representative schematic diagram of an FSL construct (FSL-1255). The function-spacer-lipid (FSL) construct consist of a lipid phosphate moiety (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) conjugated to the spacer (carboxymethylglycine) which is conjugated via a cysteine SH group to the variable peptide functional head (in this example the peptide is ID 1255)

2. METHODS OF SARS-CoV-2 KODECYTES – PREPARATION & SEROLOGICAL USE

METHOD – PREPARATION OF KODECYTES

NOTES:

- A. See Application Note #1 for safety issues.
- B. The method below is suitable for use when volumes of up to 2 mL packed cell kodecytes need to be prepared. If greater volumes of kodecytes are required, 1 mg/mL stock solution rather than 0.2 mg/mL can be prepared and dilutions adjusted accordingly.
- C. The concentration of FSLs to be used is yet to be optimized and depends on the serology platform used. The method below is a base method and requires further development. The example given below for the preparation of 1147+1255-1.5+2.5 kodecytes gave good specificity and sensitivity in the platform used in preliminary testing (1).
- D. Ensure all reagents are at room temperature before use.

PREPARATION OF 1147+1255-1.5+2.5 KODECYTES

Reagents & equipment required but NOT provided (excluding optional steps)

- Vortex
 - 37°C water bath
 - Centrifuge for preparing packed cells
 - Variable dispensing pipettors
 - Red cell preservative solution
1. Allow vials to come to room temperature. Open the vials.
 2. Reconstitute the 0.2 mg FSL-1147 and FSL-1255 as a 0.2 mg/mL stock solution by the addition of 1 mL of red cell preservative solution (Application Note #2).
 3. Allow to rest for 30 minutes then vortex or sonicate for 60 seconds.
 4. Use the 0.2 mg/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. To make FSL-1147+1255-1.5+2.5 working solution, add 15 µL of FSL-1147 stock solution and 25 µL of FSL-1255 into a new clean tube.
 6. Dilute with 460 µL of red cell preservative solution to create 500 µL of a working solution containing 1.5µmol/L of FSL-1147 and 2.5µmol/L of FSL-1255.
 7. Mix the FSL-1147+1255-1.5+2.5 working solution by vortex for a few seconds
 8. Add 500 µL of washed hard-packed group O red cells (Application Note #4) to working solution to create a 50% suspension.
 9. Mix and incubate at 37°C for 120 minutes. Resuspension by gentle mixing at 60 minutes is recommended.
 10. Dilute the 50% suspension with cell preservative solution by eye to 3-5% range.
 11. Prepare natural controls such as group O red cells as above (steps 8-10) but use cell preservative solution alone.
 12. Store the suspensions at 4°C. Kodecytes will remain stable for the normal duration of unmodified cells

METHOD – MANUAL SEROLOGY

The preparation of kodecytes above will allow for approximately 200 manual serology tests.

Note that 1.5+2.5 kodecytes are NOT optimal for manual serology.

Reagents & equipment required but NOT provided (excluding optional steps)

- Red cell preservative solution
- Packed red blood cells (Group O)
- Donor plasma
- Phosphate buffered saline (137mM NaCl, 10mM Phosphate, 2.7mM KCl pH 7.4)
- AHG Poly (Anti-IgG-C3d Polyspecific)
- ID-Card LISS/Coombs AHG gel cards

1. Add 50 μ L plasma to 50 μ L kodecytes. Centrifuge 10 s (3000 rpm).
2. Read and score agglutination using appropriate grading system, see Figure 2.
3. Incubate for 60 min at 37°C and read, score agglutination.
4. Wash 3x with PBS, i.e. fill tube with PBS centrifuge 1 min 3000 rpm, decant supernatant and re-mix cell pellet.
5. Repeat a further 2 times. Add 50 μ L AHG. Centrifuge for 10 s (3000 rpm). Read and score agglutination.
6. If agglutination is greater than 0 in any of the reactions, repeat manual serology testing with kodecytes made with stronger FSL and unmodified red blood cells (i.e. with no FSL) as above.

METHOD – GENERIC ANTIGLOBULIN CAT

With due respect to methodology differences in platforms, the following is a generic methodology overview.

If testing in gel card 1% suspension kodecytes (in red cell preservative solution), the kodecytes preparation above will allow for approximately 1000 gel card serology tests.

1. Label cards and remove foil.
2. Add 50 µL (or volume specified by manufacturer) kodecytes to wells of card.
3. Add 25 µL (or volume specified by manufacturer) donor plasma to appropriate wells.
4. Incubate cards as specified by the manufacturer.
5. Centrifuge (or hold at 37°C for up to 30 min).
6. Read cards and score agglutination.
7. If agglutination is greater than 0 in any of the reactions, repeat antiglobulin gel card serology testing with unmodified red blood cells (with no FSL) as above.
8. In addition, if agglutination is greater than 0, perform saline (neutral) gel card serology testing with kodecytes

3. REFERENCES AND FURTHER INFORMATION

APPLICATION NOTES

- Note #1.** *Practice appropriate blood handling precautions.* FSL-1147 and FSL-1255 constructs present no known biological or chemical risk. MSDS sheets for FSL-1147 and FSL-1255 are available from Kode Biotech (www.kodecyte.com).
- Note #2.** Stock Solutions can be diluted in buffers containing protein. Stock solutions must 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 0.2 or 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.** 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 coding process it is important to get reproducible results on future occasions or for comparison between different institutions.

REFERENCES

1. Nagappan R, Flegel WA, Srivastava K, Williams EC, Ryzhov I, Tuzikov A, et al. COVID-19 antibody screening with SARS-CoV-2 red cell kodecytes using routine serologic diagnostic platforms. *Transfusion*. 2021. <https://doi.org/10.1111/trf.16327>
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Kode
Materials

FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1 #572257
FSL-CoV12-1255(cKFDEDDSEPVLKGVK)C-SC2-L1
#736126

IMPORTANT NOTICE. This Material Safety Data Sheet (MSDS) is prepared by Kode Biotech Materials Limited in accordance with the New Zealand Occupational Safety & Health Service, Department of Labour Guidelines for the Preparation of Material Safety Data Sheets in New Zealand. The information contained herein must not be altered or deleted. Additional information may be appended to the MSDS, but it must be marked clearly to indicate that it is not part of the original.

1. COMPANY DETAILS

Supplier Name: Kode Biotech Materials Limited CN #2153669

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Telephone: +64 9 921 9710

Emergency Telephone: +64 9 921 9708 (working hours) or +64 21 71 54 94 (24 hr)

IDENTIFICATION

2. IDENTIFICATION OF THE MATERIAL

Product Names: FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1;

FSL-CoV12-1255(cKFDEDDSEPVLKGVK)C-SC2-L1

Other Names (shorthand): FSL-CoV12-1147;

FSL-CoV12-1255

Manufacturer's Product Code: #572257 FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1;

#736126 FSL-CoV12-1255(cKFDEDDSEPVLKGVK)C-SC2-L1

United Nations Number: None allocated

Dangerous Good Class: None allocated

Hazchem Code: None allocated

Toxic Substances Schedule: Not listed

Uses: To modify the surface of cells with SARS-Cov-1 and SARS-CoV-2 blood group peptides.
For *in vitro* diagnostic and research use only

3. PHYSICAL DESCRIPTION/PROPERTIES

Appearance: White or pale yellow, non-crystalline, lyophilised powder

Odour: Negligible

Boiling Point/Melting Point: Not determined

Specific Gravity: Not determined

Vapour Pressure: Negligible

Flashpoint: Not determined

Flammability Limits: Not determined

Solubility in Water: High – disperses as a clear solution in the pH range of 6-8; precipitates on acidification

Reactivity: None known

Incompatibility (materials to avoid): Strong acidic or basic reagents, oxidants, unstable in water

Hazardous Decomposition or By-products: Not determined

Hazardous polymerisation: Will not occur

4. COMPOSITION/INFORMATION ON INGREDIENTS

General Description: FSL-CoV12(series)C-SC2-L1 constructs are comprised of identical peptides each conjugated to a carboxymethylglycine based linker in turn conjugated to an activated adipate derivative of dioleoylphosphatidylethanolamine. Molecular weights are reported as sodium salts.

| Name: | MW | CAS number | Brutto formula | Proportion |
|--|------|------------|------------------------------------|------------|
| FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1 | 4046 | | $C_{176}H_{271}N_{38}O_{61}PSNa_4$ | 100% |
| FSL-CoV12-1255(cKFDEDDSEPVKGVK)C-SC2-L1 | 3945 | | $C_{166}H_{266}N_{35}O_{61}PSNa_7$ | 100% |

Amino acid composition and sequence (purity >99%)

| | |
|--|------------------|
| FSL-CoV12-1147(cSFKEELDKYFKNHTS)C-SC2-L1 | CSFKEELDKYFKNHTS |
| FSL-CoV12-1255(cKFDEDDSEPVKGVK)C-SC2-L1 | CKFDEDDSEPVKGVK |

HEALTH HAZARD INFORMATION

5. HEALTH EFFECTS

Routes of Entry: May enter the body through ingestion, skin and eye contact. If accidentally exposed any symptoms or untoward health effects are unlikely.

Toxicity: We are not aware of any toxicity for this product. These constructs and analogues have been intravenously injected into experimental animals and no adverse consequences have been observed.

Health Hazard: We are not aware of any reported or unreported health hazards for this product

Carcinogenicity: Not listed

Signs/Symptoms of Exposure: Unknown

Medical Conditions Generally Unknown

Aggravated by Exposure:

Potential Health Hazard: Substances may not be used as drugs, cosmetics, food additives or household chemicals. For Laboratory Diagnostic or Research Use Only

Chronic: None known or expected

6. FIRST AID MEASURES

Eye: Flush with water for at least 15 minutes. If symptoms arise, seek medical attention.

Swallowed: DO NOT INDUCE VOMITING. Wash out mouth with water and give water to drink. If symptoms arise, seek medical attention.

Skin: Remove contaminated clothing. Wash affected area with soap and water for at least 15 minutes. If symptoms arise, seek medical attention.

Inhaled: Remove to fresh air. Give oxygen if breathing is difficult and seek medical attention.

First Aid Facilities: Ensure water is available at point of use.

Advice To Doctor: Treat symptomatically. There is no known or expected toxicity for this substance.

PRECAUTIONS FOR USE

7. EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure Standards: No exposure limits set

Engineering Controls: None under normal operating conditions

Personal Protection: Wear protective gloves and safety glasses when handling this product. Do not pipette by mouth.

Precautions for Safe Handling and Ventilation – not necessary

Use/ Control Measures: No special precautions required

Clean up spills with soap and water

Flammability: Combustible

SAFE HANDLING INFORMATION

8. STORAGE AND TRANSPORT

Classification: Not classified as a dangerous good

Storage: Store powder below minus 10 degrees C (protected from light).

Opened product must be stored in a desiccator below minus 10 degrees C.

Product reconstituted in saline/PBS should be kept sterile and stored for no longer than 1 month at 4 degrees C. Stored solutions should be sonicated for 60 seconds before re-use.

Transport: Transport below 25 degrees C. Can be held at 4 degrees C for up to one month in temporary storage.

Stability: Unopened vials are expected to be stable as powder for 2 years at below minus 10 degrees C.

Opened vials are expected to be stable as powder for >12 months if stored dry (desiccated) and at below minus 10 degrees C.

Reconstituted solutions (2 mg/mL) are expected to be stable for 24 months if stored at below minus 10 degrees C.

Product reconstituted in water or aqueous buffers of pH 8 and above are unstable

Stability data is not yet available for storage of reconstituted product in frozen state.

Subsidiary Risk: None allocated

Packing Group: None allocated

Hazchem Code: None allocated

9. SPILLS AND DISPOSAL

Minor Spills:

- Wear protective gloves and safety glasses.
- Soak up spills with absorbent material i.e. paper towels or vermiculite.
- Place spilled material in clean, dry, sealed container for disposal.
- Decontaminate area with 1% sodium hypochlorite in water.

Major Spills:

- Wear protective gloves and safety glasses.
- Contain and absorb spills using earth, sand or inert absorbent.
- Prevent material entering open drains and waterways.
- Collect residues and seal in labelled drums for disposal.
- Decontaminate area with 1% sodium hypochlorite in water.

Disposal: In accordance with state land and waste management authority. For good environmental practice avoid discharge to waterways.

10. FIRE/EXPLOSION HAZARD

Fire/Explosion Hazard: Combustible, not explosive

11. ECOLOGICAL INFORMATION AND DISPOSAL CONSIDERATIONS

Ecotoxicity: None known. Unlikely to be harmful to the environment.

Environmental Fate: Degraded by micro-organisms

12. REGULATORY INFORMATION

Poisons Schedule Number: Not scheduled

Dangerous Good Class: None allocated

Subsidiary Risk: None allocated

Packing Group: None allocated

Hazchem Code: None allocated

13. CONTACT POINT

Further Information: Call +64 9 921 9708 or +64 9 921 9710 during working hours or +64 21 71 5494 after hours

Website Information: Further information is available at www.kodebiotech.com

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14. DOCUMENT CONTROL

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