

# beadLAMP for SARS-CoV-2 detection using QuickExtract crude lysate (for hospital)

---

## Introduction

This protocol describes the sensitive detection of SARS-CoV-2 using beadLAMP, a combination of *Reverse Transcription Loop-mediated Amplification* (RT-LAMP) with an upfront magnetic bead-based RNA enrichment step from crude patient samples inactivated in QuickExtract DNA solution. The protocol was established at the Vienna Bio Center as part of the Vienna Covid19 Detection Initiative (VCDI). Detailed findings covering the method establishment and experimental validation can be found in our pre-print '**A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing**' (<https://www.biorxiv.org/content/10.1101/2020.06.23.166397v2>)

Bei Fragen zum Protokoll oder Methode, kontaktieren sie bitte folgende Person:

Julius Brennecke  
Andrea Pauli  
Max Kellner  
Jakob Schnabl  
Julian Ross  
Dominik Handler  
Martin Matl

### Several key considerations before performing this protocol:

A) SARS-CoV-2 is a highly contagious virus and requires personal protection with appropriate safety standards. Users must follow Biosafety level guidelines outline by the CDC <https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html>

B) Separate working areas for sample preparation, reaction set-up and analysis are highly recommended. Cleaning work surfaces and pipettes with 5-10% Bleach solution (Caution)

C) Do not open RT-LAMP reactions in order to prevent carry-over cross contamination leading to false positives. The colorimetric master mix from NEB (M1804L) already contains dUTP and UDG to prevent cross-contamination and lowers the risk of reaction contamination.

D) 96-well plates must be properly sealed. This is absolutely crucial to prevent cross-well contamination. Proper plate seals (e.g. aluminium seals), a proper plate-roller (e.g. BioRad) or even better a PCR plate sealer (e.g. BioRad PX1 PCR plate sealer) can help prevent cross-well contamination.

E) Store reaction components at the appropriate temperatures outline by the manufacturer. When thawing the NEB WarmStart LAMP kit, we advise thawing at room temperature for 10 minutes with vortex mixing for 5 seconds every 5 minutes. This ensures that any Magnesium sulfate precipitate goes into solution, which is essential for proper HNB colorimetric LAMP detection and proper performance. Check Mastermix after thawing to ensure no precipitate is visible.

F) Colorimetric and fluorescent detection can be performed in the same reaction by simple addition of both dyes (the colorimetric kit from NEB already contains PhenolRed for colorimetric read-out). We found that 1-2  $\mu$ M SYTO-9 fluorescent dye to be comparable to the commercial fluorescent dye sold with NEB's WarmStart LAMP Kit. Alternative fluorescent dyes are SYTO-82. Do not use SYBR I or Eva Green as those were shown to inhibit LAMP reactions.

G) When working with crude samples lysed in QuickExtract, keep samples on ice whenever possible. While we have observed complete inactivation of nucleases after 5 minutes at 95°C, we found considerable RNase activity in biological sample types such as saliva and gargle. Due to the FBS content in Universal Transport Media (UTM), nasopharyngeal swabs in this medium may also contain RNases.

H) RT-LAMP uses 6 primers, two inner loop primers (FIP, BIP), loop primers (LF, LB) and outer bumping primers (F3, B3). A reaction contains all six primers if a single amplicon is tested. It is possible to add primers for 2 amplicons (12 primers) in a single reaction - in this case GuHCl needs to be added. Testing for 2 amplicons increases robustness and also slightly sensitivity of the reaction.

I) Primer choice:

We recommend As1 (ORF1ab) as the 'safest amplicon' particularly for hospital settings since

- it does not overlap a CDC amplicon used in the Roche Cobas assays
- it has performed extremely well in our tests regarding false-positives (no false positives)
- BUT: it is not the most sensitive primer combi we have for LAMP

The most sensitive option for beadLAMP with the colorimetric (PhenolRed) kit and Guanidinium-HCl is the double-combination of As1 and E1 primers.

Disadvantage: The E1 amplicon overlaps the RT-qPCR Cobas E gene assay, thus there is a risk of cross-contamination;

#### Primer sequences are

As1\_F3

CGGTGGACAAATTGTCAC

As1\_B3

CTTCTCTGGATTTAACACACTT

As1\_LF

TTACAAGCTTAAAGAATGTCTGAACACT

As1\_LB

TTGAATTTAGGTGAAACATTTGTCACG

As1\_FIP

TCAGCACACAAAGCCAAAAATTTATCTGTGCAAAGGAAATTAAGGAG

As1\_BIP

TATTGGTGGAGCTAAACTTAAAGCCCTGTACAATCCCTTTGAGTG

E1-F3

TGAGTACGAACTTATGTACTCAT

E1-B3

TTCAGATTTTTAACACGAGAGT

E1-FIP

ACCACGAAAGCAAGAAAAAGAAGTTCGTTTCGGAAGAGACAG

E1-BIP

TTGCTAGTTACTAGCCATCCTTAGGTTTTACAAGACTCACGT

E1-LB

GCGCTTCGATTGTGTGCGT

E1-LF

CGCTATTAACATTAACG

## Materials

### › Materials

- › QuickExtract DNA solution 50ml (Lucigen [QE09050](#)), store at -20°C
- › AmpureXP RNA beads (A63987), store at 4°C
- › Bead dilution buffer (2.5 M NaCl, 10 mM Tris-HCl pH 8.0, 20% (w/v) PEG 8000, 0.05% Tween 20, 5 mM Na<sub>3</sub>N) (**CAUTION with Na<sub>3</sub> read MDS**), store at 4°C
- › WarmStart® Colorimetric LAMP 2X Master Mix with UDG (NEB [M1804L](#)), store at -20°C
- › SYTO™ 9 Green Fluorescent Nucleic Acid Stain, 5mM (2500x to 5000x), (Thermofisher S34854), store at -20°C under light protected conditions
- › Primer sets E1 (SARS-CoV-2 E-gene), As1 Rabe&Cepko (SARS-CoV-2 Orf1ab-gene), sequences are outlined above, store at -20°C

- › HBSS - Hank's Balanced Salt Solution without Phenol (Thermofisher [14025092](#))
- › Nuclease free water (not DEPC-treated) 100ml, (Thermofisher [AM9938](#))
- › Guanidine hydrochloride solution, 8M (Sigma, 50937-100ML) (only needed if performing double-amplicon reactions with As1 and E1)
- › Magnet for separation of magnetic beads in PCR plates (96-well) or PCR tubes such as DynaMag (Thermofisher [492025](#), or NEB NEBNext<sup>®</sup> Magnetic Separation Rack S1515S)
- › 1.5 ml Eppendorf DNA low-bind tubes or equivalent product free from RNases and DNases
- › DNase/RNase free PCR-strips or 96-well PCR plates with appropriate seal
- › Plate roller (e.g. BioRad MSR0001) or PCR plate sealer (e.g. BioRad PX1 PCR plate sealer 1814000) to ensure proper sealing of the plates
- › 2 heat-blocks (e.g. Eppendorf ThermoStat, with heated lid) or 2 PCR machines
- › Optional: Twist Synthetic SARS-CoV-2 RNA Controls Isolate MT007544.1 (Twist Bioscience 102019) for positive control testing, store stock (1e5 copies/μl) or working dilutions (1e3 copies/μl) at -80°C. Dilutions can be temporarily stored at -20°C. Avoid multiple freeze and thaw cycles.

## Procedure

### Preparation of reaction components

#### 1. 10x LAMP Primer mixes

Thaw 100μM Primer stock and make 100μl of 10x primer mixes. Larger volumes are made by scaling up volumes of individual components

| 10x LAMP primer mixes |                     |                          |                           |             |
|-----------------------|---------------------|--------------------------|---------------------------|-------------|
|                       | A                   | B                        | C                         | D           |
| 1                     | Reagent             | amount to add for E1 mix | amount to add for As1 mix | Comments    |
| 2                     | F3 (100uM stock)    | 2                        | 2                         | 2μM at 10x  |
| 3                     | B3 (100uM stock)    | 2                        | 2                         | 2μM at 10x  |
| 4                     | LF (100uM stock)    | 4                        | 4                         | 4μM at 10x  |
| 5                     | LB (100uM stock)    | 4                        | 4                         | 4μM at 10x  |
| 6                     | FIP (100uM stock)   | 16                       | 16                        | 16μM at 10x |
| 7                     | BIP (100uM stock)   | 16                       | 16                        | 16μM at 10x |
| 8                     | Nuclease free water | 56                       | 56                        |             |
| 9                     | Total (μl)          | 100                      | 100                       |             |

#### 2. Prepare 50x Syto9 solution in DMSO

**CRITICAL**      **Store solution under light protected conditions.**

| Syto-9 dye stock |                    |               |
|------------------|--------------------|---------------|
|                  | A                  | B             |
| 1                | Reagent            | amount to add |
| 2                | Syto9 stain<br>5mM | 2             |
| 3                | DMSO               | 198           |
| 4                | Total (μl)         | 200           |

### 3. Prepare 1:5 dilution of AmpureXP RNA beads

**CRITICAL** Equilibrate AmpureXP RNA beads and Bead dilution buffer at room temperature for 30 minutes prior to use. We further recommend fresh dilution of beads and to avoid using dilution that have been stored for more than 3 days. The mixture below is sufficient for 100 bead-LAMP reactions using 60μl of diluted bead solution per reaction

| AmpureBeads Mix |                           |               |
|-----------------|---------------------------|---------------|
|                 | A                         | B             |
| 1               | Reagent                   | amount to add |
| 2               | AmpureXP RNA beads (μl)   | 1200          |
| 3               | Bead dilution buffer (μl) | 4800          |
| 4               | Total (μl)                | 6000          |

## Sample inactivation and lysis using Lucigen QuickExtract DNA solution

4. Thaw an aliquot of QuickExtract DNA solution on ice and vortex before use. (50μl per patient)
5. On ice, mix 50μl of original sample material (nasopharyngeal swab, gargle or saliva) with 50μl of QuickExtract solution in PCR strips or in a 96-well plate; seal the plate (or close the PCR strips with the lids)

**CRITICAL** Potentia

6. Heat-inactivate QuickExtract:Sample mix at 95°C for 5 minutes, then cool on ice or store at -80°C

**PAUSE** Sample can be stored at -80°C at this point for up to four weeks, potentially longer

## Bead-LAMP

7. Prepare the bead-LAMP reaction mix

If multiple reactions are performed, we strongly recommend to prepare a Mastermix for all reactions including a 10% excess. For example, when intending to run 10 reactions, prepare a Mastermix for 11 total reactions. This minimises pipetting errors and ensures enough reagent mixture to be distributed over individual PCR tubes or PCR wells.

**CRITICAL** **If performing only colorimetric based detection, the fluorescent dye can be replaced with the same volume of nuclease-free water. Syto-9 can be added to record real-time fluorescent kinetic profiles and end-point fluorescence. This is especially useful when setting up the technique and optimising reaction conditions such as primers and sample types.**

| MasterMix set-up for single (As1) amplicon |                         |                             |  |   |
|--|-------------------------|-----------------------------|--|---|
|  | A                       | B                           | C  | D |
| 1  | Reagent                 | per 20µl bead-LAMP reaction | Mastermix for 10 bead-LAMP reactions (+ 10%) |   |
| 2  | WarmStart LAMP Kit (2x) | 10                          | 110  |   |
| 3  | Primer mix As1 (10x)    | 2                           | 22   |   |
| 4  | Nuclease free water     | 7.6                         | 83.6   |   |
| 5  | Syto9 (50µM) (optional) | 0.4                         | 4.4  |   |
| 6  | <b>Total</b>            | <b>20</b>                   | <b>220</b>                                   |   |
| 7  |                         |                             |  |   |

For performing bead-LAMP using the double-amplicon combi of As1 and E1 and GuHCl, prepare the following mix:

| MasterMix set-up for double (As1+E1) amplicon + GuHCl |                                      |                             |  |   |
|---|--------------------------------------|-----------------------------|--|---|
|   | A                                    | B                           | C  | D |
| 1   | Reagent                              | per 20µl bead-LAMP reaction | Mastermix for 10 bead-LAMP reactions (+ 10%) |   |
| 2   | WarmStart colorimetric LAMP Kit (2x) | 10                          | 110  |   |
| 3   | Primer mix As1 (10x)                 | 2                           | 22   |   |
| 4   | Primer mix E1 (10x)                  | 2                           | 22   |   |
| 5   | GuHCl (8M)                           | 0.1                         | 1.1  |   |
| 6   | Nuclease free water                  | 5.5                         | 60.5   |   |
| 7   | Syto9 (50µM) (optional)              | 0.4                         | 4.4  |   |
| 8   | <b>Total</b>                         | <b>20</b>                   | <b>220</b>                                   |   |
| 9   |                                      |                             |  |   |

8. To 100µl of QuickExtract Lysate in PCR tubes, add 60µl of diluted AmpureXP RNA beads (0.6x of lysate volume), mix and incubate for 5 minutes at room temperature

**CRITICAL** If real-time fluorescent signal will be measured, transfer lysate to a PCR plate or PCR tubes suitable for real-time measurement. All downstream enrichment and detection steps are performed on this plate/tube.

**CRITICAL** For volumes smaller than 100µl, it is important to bring the volume up to 100µl with HBSS before addition of 60µl diluted beads. We have successfully used initial lysate volumes of 40-100µl.

9. After 5 minutes, place mixtures containing samples and beads onto a magnetic rack for 5 minutes to allow for complete separation of beads from the solution

**CRITICAL** It is important to await complete separation to avoid loss of beads. This is usually complete after 5 minutes and should be visible by accumulation of beads at the magnet-tube interphase.

10. Without disturbing the beads, carefully remove the solution while keeping the tubes on the magnetic rack.

**CRITICAL** It's important not to disturb the beads as the nucleic acid material is bound to it and would be lost

11. While keeping the tubes on the magnetic stand, add 200µl of 85% EtOH solution to the beads and wait for 30 seconds, then remove the wash solution

**CRITICAL** It's important not to disturb the beads  
It's important to remove any residual Ethanol since this can lead to a color-change of the Phenol dye from pink to yellow (without the reaction taking place)

12. Remove tubes from the magnetic rack and allow the beads to air-dry for 3-5 minutes.

**CRITICAL** Do not exceed 5 minutes to prevent full drying of the beads.

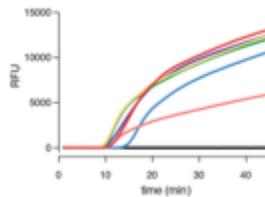
13. To each well/tube containing beads, add 20µl of LAMP reaction mixture and mix by pipetting to bring the beads into solution.

**CRITICAL** Ensure that the beads went into solution. The mix should appear brownish with no transparent phase

**CRITICAL** If real-time fluorescence is recorded, transfer reaction to a suitable detection vessel (qPCR plate)

14. Transfer the reaction to a suitable stable heat-source such as a qPCR/PCR thermocycler or heat-block and run reactions at 63°C for 30 minutes.

For real-time fluorescence data acquisition of beadLAMP in a qPCR thermocycler, we typically perform 30 cycles at 63°C with 1-minute cycle length and reading at the end of each cycle. SYTO-9 or NEB fluorescent Dye require data acquisitions using a standard FAM filter (494nm/518nm absorption/emission).



After 30 minutes run-time, remove reactions and allow to cool briefly at room temperature. Then proceed to inspect reactions visually.

**Negative reactions are pink (Phenol)**

**Positive reactions yellow (Phenol)**

15. **CRITICAL** Discard reactions. DO NOT open and DO NOT run on a gel