# D2PCR™ Buffer

for Direct-to-PCR applications

REF 2-030

IVD

Σ 100 Extractions

CE

2-8°C

| | | |
|---|---|---|
| 1. | **D2PCR™ Buffer** | 3x 1.75 ml |
| 2. | **Instructions For Use** | 1 |

**ViennaLab Diagnostics GmbH**
Gaudenzdorfer Guertel 43-45
A-1120 Vienna, Austria
Phone: (+43-1) 8120156-0
info@viennalab.com

ViennaLab®

www.viennalab.com

# PLEASE NOTE

A direct-to-PCR approach is not applicable for all RealFast™ Assays.
The **D2PCR™ Buffer** must not be used for the following applications:

| **RealFast™ Assays** | **REF** |
|---|---|
| CAH RealFast™ CNV Assay | 7-410 |
| CYP2D6 RealFast™ CNV Assay | 7-420 |

For the above listed assays, DNA extraction has to be carried out as recommended in the Instructions For Use of the respective assay.

# Instructions for use

## I. INTENDED USE

Sample dilution buffer designed to be used for ViennaLab RealFast™ Assays in a direct-to-PCR approach. *For human in vitro diagnostics.*

## II. MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to standard molecular biology laboratory equipment, the following is needed:

- Adjustable microcentrifuge capable of ≥ 500 x g (optimal 12,000 x g)
- Incubator (e.g. heating block, thermocycler) capable of 98°C (± 0.5°C)
- Microtubes (1.5 ml with screw cap) or PCR vessels (0.2 ml)

## III. ASSAY PROCEDURE

*Use fresh or frozen blood with EDTA anticoagulant only; avoid blood containing heparin or citrate. Do not store blood for more than 3 days at ambient temperature or more than 1 week at 2-8°C before use. Blood which has been kept frozen for more than one year, or gone through more than one freeze-thaw cycle is unsuitable to be used in this procedure.*
*Bring blood samples to room temperature. Mix well by carefully inverting blood collection tubes several times. Repeat mixing each time before withdrawing an aliquot of blood.*
*Allow D2PCR™ Buffer to reach room temperature.*

- Pipette **100 µl blood sample** into a 1.5 ml microtube with screw cap

  OR optionally

  into a 0.2 ml PCR vessel (8-tube strip with caps or 96-well plate with adhesive cover).
- Incubate for **10 min.** at **98°C**.
- Immediately centrifuge for **30 sec.** at **full speed** (min. 500 x g) in a microcentrifuge.
  ⚠ *If the maximum achievable centrifugal force is in the low range, centrifugation time can be extended up to 1 min. in order to allow sufficient separation.*
- Add **40 µl D2PCR™ Buffer** to the supernatant and carefully mix several times with a pipette.
  ⚠ *Do not disrupt the pellet. Use only clear supernatant in downstream applications.*

*The resulting supernatant contains DNA template suitable for immediate use in PCR. For further storage, the supernatant should be transferred into a fresh tube and kept refrigerated (2-8°C; up to one week) or frozen at -20°C.*

*Do not use hemolytic (reddish) supernatant in RealFast™ Assays.as hemoglobin will interfere with PCR efficiency.*

## IV. USE OF D2PCR™ BUFFER FOR RealFast™ ASSAYS

DNA template prepared using D2PCR™ Buffer is compatible with all Genotyping and Variant Detection RealFast™ Assays under the conditions described in the respective Instructions For Use. DNA concentration in a D2PCR™ Buffer supernatant is generally lower than in conventional preparations. Accordingly, **Controls** supplied with RealFast™ Assays have to be **diluted 1:50 with D2PCR™ Buffer** prior to use.

### Data analysis and interpretation of results

For interpretation of results follow the Instructions for use of the respective RealFast™ Assay. Due to low DNA amounts in direct-to-PCR preparations, particularly those in 96-well PCR plates, late amplification of samples may occur (i.e. beyond the threshold level of $C_q$ > 37). Thus, PCR cycles can be increased up to 45 for these samples.

For valid results, direct-to-PCR samples must cross the threshold line before $C_q$ 40. Samples with a $C_q$ > 40 should be repeated.

⚠ *In heterozygous samples both amplification curves have to raise from baseline at the same cycle number, but may result in different levels of fluorescence during the course of amplification. This is of no impact for the validity of results.*

### Fast Mode Cycling on the MIC qPCR Cycler

Use of the MIC qPCR Cycler (Bio Molecular Systems) allows running ViennaLab Genotyping and Variant Detection RealFast™ Assays with an ultrarapid cycling program. In this case the temperature profile has to be changed according to the following settings:

| Cycles | Temp | Time | Steps |
|---|---|---|---|
| 1 | 95°C | 3 min | Initial denaturation |
| 40 | 95°C | **5 sec** | Denaturation |
| | 60°C | **5 sec** | Annealing / Extension<br>Data acquisition on Green and Yellow (singleplex assay)<br>or<br>Green, Yellow, Orange and Red (multiplex assay) channels |

Fast Mode Cycling on the MIC qPCR Cycler can be used for all RealFast™ Assays except for those listed on p. II, as well as the following mpx RealFast™ Assays:

AAT mpx RealFast™ Assay (REF 7-265 / 7-268)
CYP2C9 mpx RealFast™ Assay (REF 7-225 / 7-228)

Irrespective of DNA preparation method, these assays have to be run using the PCR programs outlines in their respective Instructions For Use.

## V. QUALITY CONSIDERATIONS

- A thorough understanding of the procedure outlined here, and precise laboratory equipment and techniques are required to obtain reliable results. Use of D2PCR™ Buffer for human *in vitro* diagnostics needs to be limited to appropriately trained personnel.
- Do not use D2PCR™ Buffer beyond the expiration date printed on the outside of the kit box. Do not mix reagents from different lots.
- Avoid microbial contamination and cross-contamination of reagents or samples by using sterile disposable pipette tips throughout. Do not interchange bottle caps.

## VI. SAFETY

- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and disposable gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with clinical specimens in a biohazard waste container.
- Adhere to all local and federal safety and environmental regulations which may apply.

## VII. TROUBLESHOOTING

Advise on troubleshooting may be obtained by contacting ViennaLab through the local distributor or directly at techhelp@viennalab.com.

REF

Σ

| | | |
|---|---|---|
| 2-014 | GEN$^{X}$TRACT™ Blood DNA Extraction System | 100 extractions |
| 2-020 | Spin Micro DNA Extraction Kit | 20 extractions |
| 2-030 | D2PCR™ Buffer | 100 extractions |

Distributed by:

Manufacturer:

**ViennaLab Diagnostics GmbH**
Gaudenzdorfer Guertel 43-45
A-1120 Vienna, Austria
Phone: (+43-1) 8120156-0
info@viennalab.com

ViennaLab®

www.viennalab.com