Direct PCR without DNA extraction - which samples, and how to set them up

A practical guide to running the DBdirect™ PCR & qPCR mixes straight from crude sample.

DBdirect™ mixes combine an inhibitor-resistant **DB SuperSens Taq** polymerase with an enhancer-stabilized, single-component formulation, so DNA is amplified **directly from cells, viruses and biological matrices without a separate extraction or purification step**. Two handling routes cover the various range of sample types: add the sample **directly to the mix** or perform a fast pre-treatment using **Lysis Buffer A** (DB-1281). For many sample types both routes work well - the better choice often depends on the **number of cells in the reaction**, because high cell loads can inhibit the PCR. This application note summarizes each input type to the recommended route and gives the basic reaction setup.

CHOOSE YOUR ROUTE BY SAMPLE TYPE

Sample type	Examples tested	Recommended application	Notes
Mammalian cells	*Human cell line (CCRF-CEM)*	**Lysis Buffer A** *preferred; direct also works*	Both routes reach similar sensitivity, but Lysis Buffer A gives lower Ct. Direct addition can inhibit at high cell numbers - Lysis Buffer A preferred, especially for high loads.
DNA viruses non-enveloped	*Adenovirus (ADV)*	**Direct or Lysis Buffer A**	Both routes work well across a wide concentration range.
DNA viruses enveloped	*HSV-1*	**Lysis Buffer A** *preferred; direct also works*	Comparable sensitivity either way; Lysis Buffer A gives lower Ct and avoids the inhibition seen with direct addition at higher particle loads.
Gram-negative bacteria	*E. coli*	**Direct to mix**	Thin wall lyses readily; effective across a wide range. A 5-min initial denaturation is sufficient; colony-PCR compatible.
Gram-positive bacteria easy-lysing G+	*Lactobacillus*	**Direct (low load)** or **Lysis Buffer A (high load)**	Add directly for low cell numbers (≤ ~1,000 / rxn); use Lysis Buffer A for higher cell numbers (≥ ~1,000 / rxn).
Gram-positive bacteria tough wall G+	*Rhodococcus*	**Lysis Buffer A**	Rigid wall; markedly more sensitive after a 20 min / 95 °C Lysis Buffer A pre-treatment.
Yeast / fungi	*S. cerevisiae, Candida*	**Lysis Buffer A**	Rigid wall needs pre-treatment.

Recommended route reflects an internal comparison of Lysis Buffer A pre-treatment vs. direct addition using probe qPCR with species-specific primers/probes, 20 µL reactions, 1 - 400,000 cells or viral particles per reaction. Establish your own limit of detection for each organism–matrix combination.

SENSITIVITY BY MIX

Organism / virus	Direct to the mix	Lysis Buffer A pre-treatment
Human cells	+++	+++
G– bact. (*E. coli*)	+++	+++
G+ bact. (*Lactobacillus*)	+++	++
G+ bact. (*Rhodococcus*)	++	++
Yeast (*S. cerevisiae*)	+	+++
Non-env. virus (ADV)	+++	+++
Env. virus (HSV-1)	+++	+++

Approx. lowest input reliably detected: +++ 1–40 ++ 100–4,000 + 10,000–400,000 cells or particles / rxn.

BASIC REACTION SETUP

Component (20 µL reaction)	Amount
DBdirect™ PCR Mix (2X)	10 µL
Forward + reverse primer	250-1000 nM each
Probe(s) *optional*	100-250 nM each
Sample / Lysis Buffer A lysate	up to 5 µL
PCR-grade water	to 20 µL

What matters most is the **final number of cells / particles in the reaction**, not the sample volume - high loads can inhibit PCR reaction.
Aim for ~1,000–100,000 to start and titrate.

RULES OF THUMB

- **Start around 1,000 cells/particles per reaction** to check whether an organism is direct-PCR compatible.
- **What counts is the final number of cells/particles in the reaction,** not the sample volume; high loads can inhibit the reaction.
- **Default concentration of primers 400 nM, probe 200 nM -** if not, optimizing.
- **We recommend to validate for your organism with a dilution series** (e.g. 100 - 1,000 - 10,000 - 100,000 per reaction).

WORKFLOW - THREE STEPS, NO EXTRACTION

STEP 1 · PREPARE SAMPLE

Suspend cells or virus, or take crude matrix (serum, saliva, media). No pre-treatment needed for the direct route.

STEP 2 · LYSIS BUFFER A (*OPTIONAL*)

For yeast, tough Gram-positives, human cells, enveloped viruses and any high cell load:
20 min at 95 °C in DB Lysis Buffer A (DB-1281), then add up to 5 µL lysate.
Skip for Gram-negatives, non-enveloped viruses and low cell numbers.

STEP 3 · ADD MIX & RUN PCR

Combine with the DBdirect™ mix and cycle. The UDG (*optional*) + 95 °C start also lyses many inputs and prevents carry-over contamination.

CYCLING PROTOCOL

Step	Temp	Time
UDG decontamination (1×) Applies **only** to mixes with UDG	25 °C	2 min
Initial denaturation (1×) 2 min after Lysis Buffer A | 20 min direct	95 °C	2 / 20 min
Denaturation	95 °C	5 s
Annealing (+ data acquisition step)	60 °C†	15 s
Extension	72 °C	15 s
Cooling / hold (1×)	4 °C	∞

40-45 cycles

Repeat 40–45 cycles (30–35 for Gel mixes).
†Adjust to primers; do not drop below 55 °C.
Extend ≥1 min/kb for amplicons >250 bp.
E. coli: 5-min initial denaturation is sufficient.

FEATURES OF DBdirect™ PCR MIXES

- **Inhibitor resistance** - DB SuperSens Taq tolerates crude-sample and matrix inhibitors that stall standard polymerases.
- **Ultrasensitive** - detection down to ~1 copy of DNA; single-cell detection possible.
- **Built-in lysis** - the 95 °C hot-start releases DNA from many inputs' matrices without a separate step.
- **One tube** - single-component 2X mix; just add primers/probe(s) and sample.

CONCLUSION

For cells with thick cell walls (e.g. yeast, Gram-positive bacteria), human cells, and enveloped viruses, **pre-incubation with (DB-1281) DB Lysis Buffer A is recommended**.

For Gram-negative bacteria (e.g. *E. coli*) and non-enveloped viruses (e.g. ADV), **direct addition to the PCR mix is effective across a wide range of sample concentrations**.

The PCR mix method is in principle compatible with all tested cell types and viruses; however, **inhibition was observed for some microorganisms at higher concentrations**.

ORDERING INFORMATION

Catalogue number	Product name	Sizes
DB-1308	DBdirect™ PCR Probe Mix SuperSens 2.0	100 rxns | 1000 rxns
DB-1309	DBdirect™ PCR Probe Mix SuperSens 2.0 UDG	100 rxns | 1000 rxns
DB-1310	DBdirect™ PCR SYBR Mix SuperSens 2.0	100 rxns | 1000 rxns
DB-1316	DBdirect™ PCR SYBR Mix SuperSens 2.0 UDG	100 rxns | 1000 rxns
DB-1311	DBdirect™ PCR Gel Mix SuperSens 2.0	100 rxns | 1000 rxns
DB-1317	DBdirect™ PCR Gel Mix SuperSens 2.0 UDG	100 rxns | 1000 rxns
DB-1281	DB Lysis Buffer A	5 mL