Protocol for Real-Time RT-PCR of DenV 1 with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses

Isabelle Leparc-Goffart, Meili Baragatti, Sarah Temmam, Anne Tuiskunen, Gregory Moureau, Rémi Charrel, Xavier de Lamballerie

Journal of Clinical Virology 45 (2009) 61–66

PMID : 19345140

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- IVT RNA Tropic 1

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P DenV 1	6	96+5

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

  - Add 707 µl of Water molecular grade

  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 400 µL-volume

  - Rehydrated P&P must be incubated at room temperature for 10 min after which

  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	101
H2O (µL)	707

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 45sec – Plate read

5 : GOTO3, 45 more time

Protocol for Real-Time RT-PCR of Chik DUO with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Chikungunya Fever in Travelers Returning to Europe from the Indian Ocean Region, 2006

Marcus Panning, Klaus Grywna, Marjan van Esbroeck, Petra Emmerich, Christian Drosten

PMID : 18325256

Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses

Boris Pastorinoa, Mael Bessaud, Marc Grandadama,b, Severine Murric, Hugues J. Toloua,b, Christophe N. Peyrefitte

PMID : 15664052

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- ArRNA Chik DUO

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P Chik DUO	21	48+3

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

  - Add 357 µl of Water molecular grade

  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 200 µL-volume

  - Rehydrated P&P must be incubated at room temperature for 10 min after which

  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	51
H2O (µL)	357

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 45sec – Plate read

5 : GOTO3, 45 more time

Protocol for Real-Time RT-PCR of DenV 2 with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses

Isabelle Leparc-Goffart, Meili Baragatti, Sarah Temmam, Anne Tuiskunen, Gregory Moureau, Rémi Charrel, Xavier de Lamballerie

Journal of Clinical Virology 45 (2009) 61–66

PMID : 19345140

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- IVT RNA Tropic 1

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P DenV 2	6	96+5

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

  - Add 707 µl of Water molecular grade

  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 400 µL-volume

  - Rehydrated P&P must be incubated at room temperature for 10 min after which

  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	101
H2O (µL)	707

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 45sec – Plate read

5 : GOTO3, 45 more time

Protocol for Real-Time RT-PCR of DenV 3 with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses

Isabelle Leparc-Goffart, Meili Baragatti, Sarah Temmam, Anne Tuiskunen, Gregory Moureau, Rémi Charrel, Xavier de Lamballerie

Journal of Clinical Virology 45 (2009) 61–66

PMID : 19345140

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- IVT RNA Tropic 1

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P DenV 3	6	96+5

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

  - Add 707 µl of Water molecular grade

  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 400 µL-volume

  - Rehydrated P&P must be incubated at room temperature for 10 min after which

  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	101
H2O (µL)	707

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 45sec – Plate read

5 : GOTO3, 45 more time

Protocol for Real-Time RT-PCR of DenV 4 with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

"In-House" DenV 4 specific system

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- IVT RNA Tropic 1

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P DenV 4	6	96+5

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

    - Add 707 µl of Water molecular grade

    - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 400 µL-volume

    - Rehydrated P&P must be incubated at room temperature for 10 min after which

    - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	101
H2O (µL)	707

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 45sec – Plate read

5 : GOTO3, 45 more time

Protocol for Real-Time RT-PCR of DenV All DUO with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses

Isabelle Leparc-Goffart, Meili Baragatti, Sarah Temmam, Anne Tuiskunen, Gregory Moureau, Rémi Charrel, Xavier de Lamballerie

Journal of Clinical Virology 45 (2009) 61–66

Early diagnosis of dengue in travelers: Comparison of a novel real-time RT-PCR, NS1 antigen detection and serology

Eili Huhtamo, Essi Hasu, Nathalie Y. Uzcátegui, Elina Erra, Simo Nikkari, Anu Kantele, Olli Vapalahti, Heli Piiparinen

Journal of Clinical Virology 47 (2010) 49–53

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- ArRNA DenV All DUO

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P DenV All DUO	11	96+5

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

  - Add 707 µl of Water molecular grade

  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 400 µL-volume

  - Rehydrated P&P must be incubated at room temperature for 10 min after which

  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	101
H2O (µL)	707

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 45sec – Plate read

5 : GOTO3, 45 more time

Protocol for Real-Time RT-PCR of ZIKA DUO with P&P

For RUO (Research Use Only)

Caution: The vials containing the primers and probe mix (P&P) should be stored after reception at -20°C in the dark. Stable at least 6 months in the described conditions.

NB: Probe: FAM-TAMRA

Assay optimization for molecular detection of Zika Virus

Victor M Corman, Andrea Rasche, Cecile Baronti, Souhaib Aldabbagh, Daniel Cadar, Chantal BEM Reusken, Suzan D Pas, Abraham Goorhuis, Janke Schinkel, Richard Molenkamp, Beate M Kümmerer, Tobias Bleicker, Sebastian Brünink, Monika Eschbach-Bludau, Anna M Eis-Hübinger, Marion P Koopmans, Jonas SchmidtChanasit, Martin P Grobusch, Xavier de Lamballerie, Christian Drosten & Jan Felix Drexler

PMID : 27994281

"In-House" ZIKV specific system

Reagents with which the assay has been validated at UVE (Unité des Virus Emergents, Marseille, France):

- SuperScript™ III Platinum™ One-Step qRT-PCR Kit, ThermoFischer ref 11732-088

- Water molecular grade

- ArRNA Zika DUO

1. *Kit contents :*

Nom	Number of vials	Number of assays/vial
QC_Lyoph-P&P Zika DUO	40	24+2

Table 1. List of products in the kit

1. Rehydration of Lyoph-P&P before use (Table 1)

- Write the date on the vial before opening. - Lyoph-P&P is respudended as described:

  - Add 182 µl of Water molecular grade

  - Homogenization by 10 to 20 times pipetting up and down in the glass vial a 100 µL-volume

  - Rehydrated P&P must be incubated at room temperature for 10 min after which

  - A second step of 10 times multiple pipetting must be done.

WARNING: These steps are critical to ensure adequate homogenization

Number of tests/vial	26
H2O (µL)	182

Table 2. Lyoph-P&P regeneration; rehydrated Lyoph-P&P can be stored at 4 °C for up to 7 days

*** this volume is adapted to the SuperScript™ III Platinum™ One-Step qRT-PCR Kit and can vary depending upon the RT-PCR kit**

2. *Preparation of the reaction mix*

MasterMix	25µL Single rxn, µL
2X Reaction mix*	12,5
Rehydrated Primers and probe P&P**	7
SSIII/Taq Enzyme Mix*	0,5
Total	20
Template RNA	5

*, ThermoFischer / Invitrogen: SuperscriptIII One-Step RT-PCR system with Platinum Taq DNA Polymerase

**, as indicated in Table 2.

3. *Cycling program and RT-PCR reaction*

1 : 50°C – 15min

2 : 95°C – 2min

3 : 95°C - 15sec

4 : 60°C - 30sec – Plate read

5 : GOTO3, 45 more time