



## **SalivORAL DNA/RNA extraction kit**

*Methods and products specially designed for saliva analysis*

### **Adaptative range to your needs**

- A kit for collecting and stabilizing stimulated saliva,*
- A kit for extracting and purifying total nucleic acids (ADN+ARN),*
- A kit to analyzed volatiles compounds (VOCs) by SPME-GC-MS.*



## SalivORAL range products

- **Saliva collection kit : SaliCOL**

Kit contains :

- ✓ a tartrazine solution that stimulates salivation,
- ✓ a beaker in which the stimulated saliva is harvested
- ✓ 2 salivary sample holding tubes containing a lyophilized compound that stabilizes saliva for 10 days at room temperature



*The collected sample represents a volume of 4 to 6 ml and contains about 50% stimulated saliva and 50% salivation and stabilization solutions.*

- **Total nucleic acids extraction and purification kit : DNA/RNA GenoSPYD *Magnetic beads or column***

The purification of the total nucleic acids (DNA + RNA) is carried out on a 250  $\mu$ L sample of the salivary sample. The nucleic acids, after extraction and purification, are concentrated in a volume of 50  $\mu$ L. DNase step is optional.

*The collecting method of the stimulated saliva linked with the purification method of nucleic acids makes it possible to obtain an average of  $352 \pm 38.4$   $\mu$ g of total nucleic acids.*

*GenoSPYD Total Nucleic Acid extraction is manual or automated using the IDEAL or KingFisher platforms*



The total nucleic acids purified and concentrated by this method can be analyzed by QPCR, Reverse Transcriptase Q-PCR and Digital PCR systems.

- **Stabilization kit to volatile organic compounds analysis : SaliVOC**

A stabilization kit is proposed to prepare salivary samples for VOCs analysis by SPME-GC-MS. This method was developed on a sample volume of 2.5 mL of the saliva sample.

*The method allows the identification at least 78 volatile organic compounds (208 VOCs were identified on 20 studied saliva).*



Institut Clinident has a proprietary salivary VOCs database and can offer the analytical service and/or results exploration.

**Advantages of SalivORAL range:**

- A single sampling kit to stabilize DNA, RNA and volatile compounds;
- A volume of salivary sample allowing to carry out several analyzes simultaneously;
- The same purification kit for extracting and concentrating the DNA and RNA;
- A sampling kit compatible with analyzes of volatile organic compounds by SPME/GC/MS.

**Quantitative evaluation of total nucleic acids collected**

- **kits Salicol and DNA/RNA GenoSPYD results:**

Average quantity of total nucleic acids quantified in a sample of 250 µL: **22 ± 2.4 µg**

| Average of optical density (260 nm) | Medium concentration in the assay solution (µg/µl) | Average concentration in the solution of purified nucleic acids (µg/µl) | Average amount in the stimulated saliva sample of 250 µl (µg) |
|-------------------------------------|--|---|---|
| <b>0.028 ± 0.003</b>                | <b>0.0011 ± 0.00012</b>                            | <b>0.22 ± 0.024</b>   | <b>22 ± 2.4</b>   |

Average amount of total nucleic acids that can be extracted from the total salivary sample:  
**352 ± 38.4 µg**

| Average amount in the stimulated saliva sample of 250 µl (µg) | Average amount in total of 4 ml sample of stimulated saliva (µg) |
|---|--|
| <b>22 ± 2.4</b>   | <b>352 ± 38.4</b>  |

- **Comparison of the nucleic acid level obtained with different collection methods:**

| Sampling method   | Collected volume                        | Nucleic acids rates                       |
|---|---|---|
| Cotton swab <sup>1</sup>  | 1 swab                                  | 1.9 µg d'ADN                              |
| Guthrie cards <sup>2</sup>  | 1 Carte                                 | 2.3 µg d'ADN                              |
| Cytobrush <sup>3</sup>  | 1 Cyto-brosse                           | 6.8 µg d'ADN                              |
| Mouthwash <sup>4</sup>  | 10 ml                                   | 35.1 µg d'ADN                             |
| Collection of non-stimulated saliva with Oragene kit <sup>5</sup> | 2 ml                                    | 110 µg d'ADN                              |
| Collection of stimulated saliva with salicol kit                  | 4 ml included 2 ml of stimulated saliva | 352 µg of total nucleic acids (ADN + ARN) |

<sup>1</sup> Cozier, Y., Palmer, J. and Rosenberg, L. (2003). Comparison of methods for collection of DNA samples by mail in the black women's health study. AEP. 14, 117-122

<sup>2</sup> Harty, L., Garcia-Closas, M., Rothman, N., Reid, Y., Tucker, M. and Hartge, P. (2000). Collection of buccal cell DNA using treated cards. Cancer Epidemiology, Biomarkers and Prevention. 9, 501-506.

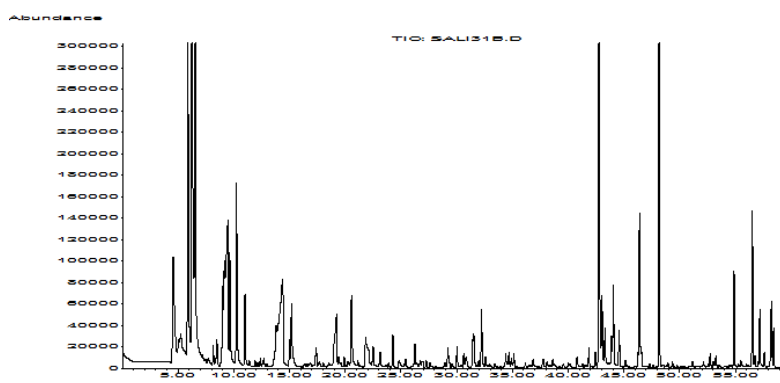
<sup>3</sup> Montserra, G., Egan, K., Abruzzo, J., Newcomb, P., Titus-Ernstoff, L., Franklin, T., et al (2001). Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiology, Biomarkers and Prevention. 10, 687-696.

<sup>4</sup> Le Marchand, L., Lum-Jones, A., Saltzman, B., Visaya, V., Nomura, A., and Kolonel, L. (2001). Feasibility of collection buccal cell DNA by mail in a cohort study. Cancer Epidemiology, Biomarkers and Prevention. 10, 701-70.

<sup>5</sup> Birnboim, H.C. DNA yield with an Oragene® self-collection kit. <http://www.dnagenotek.com/ROW/pdf/PD-WP-001.pdf>

<sup>6</sup> Institut Clinident : Données internes

### SaliVOC : Saliva volatile compounds analysis



Chromatogram of saliva sample collected with SaliCOL kit (SPME-GC-MS)

| Main chemical families | Number of identified compounds | Identified volatile compounds examples                 |
|------------------------|--------------------------------|--|
| Acid                   | 13                             | Butanoic acid, isovaleric acid ; octanoic acid ;...    |
| Alcohols               | 20                             | Ethanol ; 1-butanol-3-methyl; 3-hexanol ;...           |
| Ketones                | 40                             | 2.3-butanedione ; 2-heptanone ; cyclohexanone;...      |
| Aldehydes              | 15                             | Ethanal ; butanal-3-methyl ; nonanal;...               |
| Aromatics              | 18                             | Xylènes ; indole; phenol;...                           |
| Maillard compounds     | 15                             | Pyrazine ; Furane-2-ethyl;1H-pyrrole ;....             |
| Terpenes               | 23                             | α-pinene ; limonene ; camphene ;...                    |
| Sulfur compounds       | 6                              | Methanethiol ;dimethyl sulfide ; dimethyl disulfide;.. |

### ONCORAL™ Test

Institut clinident developed a new strategy for oral cancer risk measurement using stabilized saliva collected with SaliCOL Kit. The test combines the determination of the total flora, the search for salivary HPV and the identification of specific VOCs (Kit SaliVOC). The method and the VOC fingerprint in relation to oral cancer have been patented.

|   | ONCORAL <sup>1</sup> |
|---|----------------------|
| <b>Non-invasive collection system</b>                                     | YES                  |
| <b>Stimulated saliva collection</b>                                       | YES                  |
| <b>Sample volume</b>  | 4 à 6 ml             |
| <b>Average amount of saliva in a sample</b>                               | 2 à 3 ml             |
| <b>Sample stability at room temperature</b>                               | 10 jours             |
| <b>A single sampling kit to stabilized nucleic acids</b>                  | YES                  |
| <b>ARN purification</b>   | YES                  |
| <b>Total nucleic acids purification</b>                                   | YES                  |
| <b>Average amount in total of 2 ml saliva sample</b>                      | 352 µg               |
| <b>Sampling kit in accordance with the analysis of volatile fractions</b> | YES                  |
| <b>Sampling kit in accordance with proteomic analysis</b>                 | YES                  |

<sup>1</sup> Institut Clinident : internal data