Quick Reference Guide

# AIR-DRYING MOLECULAR ASSAYS

## BASIC WORKFLOW:

### 1 PREPARE

**Prepare reagent master mix** according to the recommended volumes illustrated in Table 2 of the *Product Handling Guides* for *MDX082* and *MDX095*. It is recommended that initial testing includes three different drying times to assess relative moisture loss. Assays should be carried out sequentially in three different drying events with identical oven settings.

### 2 WEIGH

**Weigh dry vessels** (e.g. 8-well PCR tube strips) on precision balance and record total weight (**W1** Value).

### 3 DISPENSE

**Dispense the master mix** into the vessel(s) and determine total weight using the precision balance (**W2** Value).

### 4 DRY

**Apply recommended drying conditions** as per User Guidelines for *MDX082* and *MDX095* (e.g. 20 min at 80C for MDX082 and 80 min at 50 for MDX095). Remove vessel(s) from oven and re-cap prior to recording the final weight (**W3**).

### 5 WEIGH

**Weigh final vessels** and record final weight (**W3** Value).

### 6 CALCULATE

**Calculate moisture loss:**

(Moisture Loss (%) = (W2-W3) / (W2-W1) x 100

Where:

**W1** is the weight of empty vessel (e.g. 8-well PCR tube strips)
**W2** is the weight of the vessel before drying
**W3** is the weight of the vessel after air-drying

For MDX095 the ideal moisture loss is 70% +/- 1%. Moisture loss levels above 71% or below 69% will require additional optimization using different drying times. For MDX082 the ideal moisture loss is 95% +/- 1%. Moisture loss levels above 96% or below 94% will require additional optimization using different drying times.

### 7 ANALYZE PERFORMANCE

**Performance of the dried mix** can be determined by comparing it to the equivalent wet mix in an assay using a serial dilution of the test sample. The Ct value in each dilution should be the same for both the wet and dried mixes, if the observed Ct value is lower for the dried mix, further drying optimization is required to reduce the amount of moisture loss.

### Reagents and consumables required:

- Air-Dryable™ qPCR Mix (MDX082) or Air-Dryable™ 1-Step RT-qPCR Mix (MDX095)
- Forward/Reverse Primer and Probe master mix at 20x concentration
- DNase/RNase free disposable tips for pipetting
- Vessels (e.g. 8-well PCR tube strips with caps)

### Equipment:

- Precision drying oven (temperature uniformity of +/- 1.5°C) with convection, fan-forced or vacuum capabilities (e.g. Memmert UF260Plus)
- Precision balance (readability to 0.001g)

### Additional notes:

To reduce contamination, perform RNA work using RNase-free plasticware in an RNase-free area. Keep the qPCR reaction assembly area separate from the qPCR equipment area.

To test a sample or to ask additional questions, E: **info@meridianlifescience.com**

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# FAQs

## Important Principles for Air-Drying Molecular Assays

Air-drying is an evaporation process where water is gradually removed from the material. It requires a precision oven and optimization of both the temperature and time used to achieve sufficient drying. Each mix (MDX095 and MDX082) has a specific ideal moisture loss level (71% and 95% respectively) that, once achieved, indicates drying is complete.

Guidelines for the oven temperature and drying times are provided in the product handling guides. Optimization will be required as many factors can impact the evaporation process such as the vessel used, the volume per reaction well/tube, the total volume per lot and the final formulation of the mix (with primers and probes).

**Q** Can I use the Lyo-Ready mix for air-drying?
No. Air-drying requires specific stabilizers, excipients and preservatives which are different from those required for lyophilization.

**Q** Is it possible to adjust the oven temperature and do high temperatures damage the polymerase or reverse-transcriptase?
Yes, we recommend optimizing the oven temperature and drying time for each new assay formulation (mix including primers and probes), vessel used and lot size.

High drying temperatures may affect the integrity of the mix and its components, including enzymes.

**Q** How do you know once drying is complete?
Ideally, 70% moisture should be removed from Air-Dryable™ 1-step RT-qPCR Mix (MDX095) and 95% from the Air-Dryable™ Mix (MDX082). However, the optimal moisture loss requires optimization for each assay. Retaining too much moisture may impact the shelf-life of the assay and over-drying may make the mixture difficult to rehydrate and cause a loss of performance. Moisture loss can be calculated by comparing the weight of the initial wet mix in the vessel to the dry mix (refer to the basic workflow for further information). Please note that the air-dried material must be packaged immediately after the drying cycle.

**Q** Is it easy to rehydrate an air-dried mix? Do you need to vortex?
The air-dried mix rehydrates in seconds after the addition of the sample. We do recommend, if possible to gently shake/mix the vessels in order to resuspend the reaction mix before running the reaction, or alternatively mix the solution when adding the patient sample.

**Q** Why do I sometimes see a lower Ct value when I compare the performance of the re-hydrated dry mix against the wet mix?
If a mix becomes over-dried, the integrity of the enzymes will be compromised. Conceptually, the ideal "dryness" of a mix will be the highest percentage of moisture loss achieved without losing assay performance. If the Ct value of your dry mix is lower to the comparable wet mix, then the mix has been over-dried and further optimization is required.

**Q** Do different probes affect the performance of the assay after the mix is air-dried?
We did not observe any effect of the fluorophore type (Cy5, FAM, JOE or ROX) on the performance of the mix after air-drying.

**Q** Will the air circulation from the fan in the oven cause contamination?
We did not observe any contamination during air-drying however we do recommend good laboratory cleaning practice to minimize possible environmental contamination. Examples include surface cleaning or allocating separate locations for the oven drying, qPCR reaction setup, and analysis. In addition, we recommend maintaining the oven following the cleaning instructions provided by the manufacturer.

**Q** Could the fan air bring traces of primers/probes from one tube during the drying to another tube?
We did not observe any cross contamination from fan-forced oven drying. Given the mix resides at the bottom of the vessel, it is very unlikely that cross-contamination can occur between the wells.

In addition, in accordance with good manufacturing practices, we would discourage oven drying two different types of assay in the same drying cycle.

**Q** Do I have to use 8-well PCR tube strips?
Other vessels can be used such as plates, vials or various microchips, but the drying conditions for each vessel/assay would need to be optimized. Once the ideal conditions and parameters are established, they would need to be verified during validation and scale up.

**Q** Do you have a list of ovens that you recommend?
We recommend using a precision drying oven (temperature uniformity of +/- 1.5°C) with convection, fan-forced or vacuum capabilities. In our testing, we used a Memmert UF260Plus oven with fan and air flap settings at 100% and 20% respectively. Please note that different ovens will have different specifications and adjustable parameters that will need to be optimized based your assay, reaction volume, vessel type, batch size, relative humidity, and altitude.

**Q** Can I simply put the tubes back in the oven for longer incubation if the moisture loss is not enough, or add water if it is too much?
If the first attempt is not successful and does not provide the correct moisture loss value, we do not recommend putting the tubes back in the oven for additional drying or adding water. We recommend re-starting the experiment in order to control all of the variables so that they are reproducible. In addition, opening the door during the drying process is also not recommended unless it is done in a controllable manner and will be part of the process moving forward.

**Q** Can I test using different vessel types and/or different reaction volumes per well in the same drying cycle?
We recommend that different drying cycles are performed to test the effect of altering any assay variable such as vessel type, reaction volume, primer/probes, and lot size. This will ensure that the conditions and outcomes are reproducible.

To test a sample or to ask additional questions, E: **info@meridianlifescience.com**

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