



The Pig 4MID® Kit

**A Functional Assay for Semen Quality
and Boar Fertility Assessment**

The Pig 4MID[®] Kit : a functional assay of sperm quality and boar fertility



- The **Pig 4MID[®] Kit** is a functional and molecular approach to assess semen quality
- The **Pig 4MID[®] Kit** allows to quantify the **amount of proAKAP4** in spermatozoa
- For all boar breeds
- The **Pig 4MID[®] Kit** relies on a robust ELISA assay
- The **Pig 4MID[®] Kit** is an easy and cost-saving approach to evaluate boar semen quality
- The **Pig 4MID[®] Kit** is working with fresh, chilled or frozen semen in extenders



The Pig 4MID[®] Kit : a functional assay of sperm quality and boar fertility



- The Pig 4MID[®] Kit allows to quantify the **amount of proAKAP4** in spermatozoa
- ProAKAP4 is only **expressed in the flagellum** of mature and functional spermatozoa
- ProAKAP4 and AKAP4 are **strictly required for sperm motility** and **male fertility**
- The amount of proAKAP4 is indicative **OF HOW LONG and IF** spermatozoa will remain motile and fertile over time
- ProAKAP4 is the « **FUEL** » for spermatozoa motility and functionality



The Pig 4MID[®] Kit Routine Applications:



- **Semen Quality Assessment**

High concentrations of proAKAP4 are indicative of **high quality semen**:

- With a gain of fertility (up to **5% of fertility rate increase**)
- With an increased **total newborn rate** (up to **2 piglets**)

- **Shelf-Life Assessment**

High concentration of proAKAP4 is indicative of **long-lasting use of semen doses**

Predicts **HOW LONG** spermatozoa are motile and fertile (50% decrease by D5)



- **Boar Follow-ups**

Identify and classify low and high productive boars

Early Identification of boar fertility problems in boar studs



Semen Quality Assessment Applications

- Give a number: ProAKAP4 concentrations in doses are indicative of semen quality

ProAKAP4 thresholds allow to classify semen and doses, independently of other sperm parameters

Thresholds of proAKAP4 in ng/10M of spz (total spz)	Semen Quality
Less than 10	Poor
Between 20 and 40	Good
Between 40 and 60	Very Good
Over 60	Excellent

- **High ProAKAP4 concentrations are correlated with high fertility rates**
Gain from **2% to 5% fertility rate** with doses with very good or excellent quality
- **High ProAKAP4 concentrations are predictive of an increase in total newborns**
Gain of **0.11 to 0.2 piglets per 10 ng / 10 millions of spermatozoa** increase with concentrations above 20 ng / 10 millions of spermatozoa
- **High ProAKAP4 concentrations predict better post-thaw motility and fertility with frozen sperm**

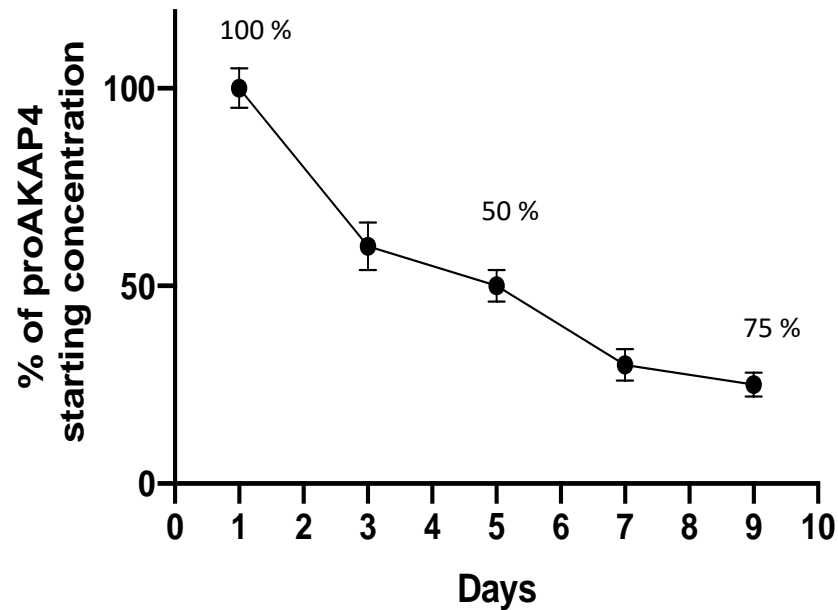
The higher proAKAP4 concentration is, the better the semen quality is !



Semen Shelf-Life Assessment Applications

ProAKAP4 concentrations are predictive of HOW LONG spermatozoa will be motile and fertile.

ProAKAP4 concentrations are TODAY the only sperm parameter able to predict **long-lasting sperm quality and motility**.



Only ProAKAP4 concentrations can predict if spermatozoa will be fertile and motile over time

Long-lasting motility is NEVER evaluated by CAS (as CASA is only a snapshot of motility)

A 50 % decrease is observed at D+5 after dose processing (all breeds and extenders dependent)

ProAKAP4 concentrations assessment with the Pig 4MID[®] Kit:

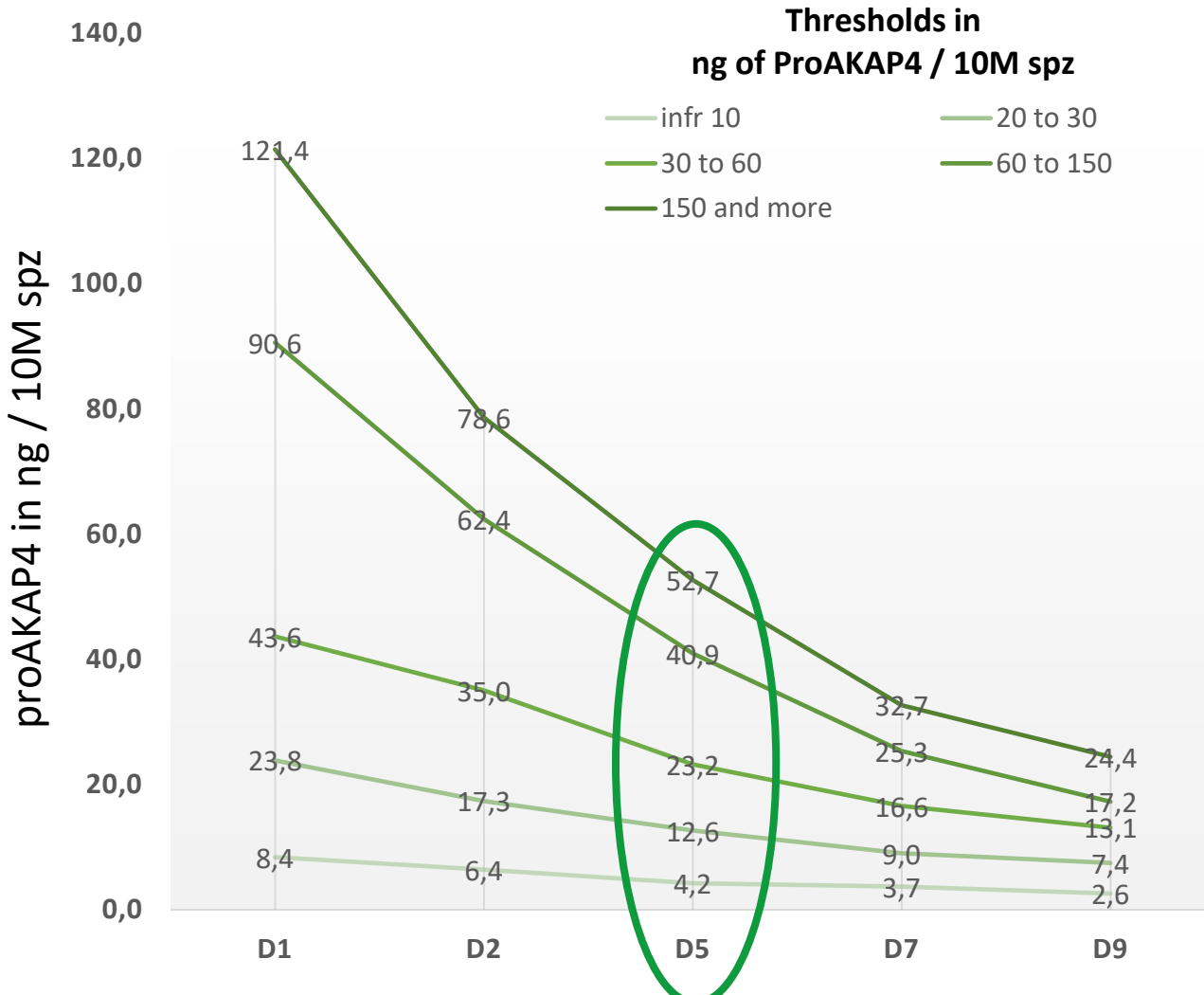
- Allows to detect long-lasting doses (marketing values)
- Optimizes production, management, use and shipment of chilled boar semen doses





Focus on Dose Semen Shelf-Life Assessment Applications of Pig 4MID® Kit

An example of shelf-life assessment of doses produced with a commercial extender, representative with individual values



From **Day 1 to Day 9**, the decrease of proAKAP4 concentration can be monitored using the Pig 4MID® Kit.

A **50 % decrease of proAKAP4** is always observed at D+5 after dose production.

The **higher the concentration of proAKAP4 at D1**, the better the sperm quality will be at D5, to ensure long-term sperm quality. Remaining amount of proAKAP4 reflects the capacity to be motile and functional at fecundation time.

Long-lasting motility is never evaluated by CASA (as CASA is only a snapshot of motility).

The amount of proAKAP4 present in ejaculated spermatozoa/dose reflects long-lasting spermatozoa functionality.



ProAKAP4 concentrations predict long-lasting preservation of sperm motility and functionality

ProAKAP4 concentrations Using the Pig 4MID [®] Kit	Total Motility CASA	Total Motility CASA	% decrease of motility
	D1	D5	
Below 40 ng/10M of spz	Over 80	Below 70	15 to 20%
40<x<60 ng/10M of spz	Over 80	Below 75	4-6%
Over 60 ng/10M of spz	Over 80	79-80	1-2%

Total motility as assessed by CASA can not be used to predict long lasting motility doses shelf-life

High concentrations of proAKAP4 at D1 will ensure maintenance of high motility at D5.

The total motility is better preserved at D5 in semen with a higher concentration of proAKAP4 as assessed the Pig 4MID[®] Kit.



In few words, to summarize about the Pig 4MID[®] Kit :



- ProAKAP4 parameter **is independent** of all the other sperm parameters presently evaluated for both sperm quality and shelf-life assessments in boar studs.
- CASA sperm parameters **cannot predict dose shelf-life** while proAKAP4 **clearly can do it :**
 - => A **50% decrease** of proAKAP4 concentration is expected at D5 and long-term use doses should be selected among ejaculate with high proAKAP4 concentration. For a shelf life of 5 days, the dose should at least have a concentration of 60 ng / 10 millions of spermatozoa.



The Pig 4MID[®] Kit :

Is today the only ELISA Kit on the market that quantify the proAKAP4 concentrations in fresh, chilled and frozen semen



- **Opportunities for use of the Pig 4MID® Kit :**

- ✓ **Identify, classify the best productive boars**
- ✓ **Optimize management, processing and shipment of doses**
- ✓ **Warrant the shelf-life using proAKAP4 concentrations at D1**
- ✓ **Select the best semen doses (marketing values)**
- ✓ **Increase the number of doses produced based on proAKAP4 thresholds (10 to 15% obtain easily without process changes)**
- ✓ **Main added values : proAKAP4 concentrations are predictive of boar fertility as well as long-time use of doses**
- ✓ **In house / boar studs**
- ✓ **Etc.**





Summary on the Pig 4MID[®] approach



- The Pig 4MID[®] approach is **easy and robust** : gives numerical semen quality values based on thresholds of proAKAP4 concentrations (**functional sperm parameter**).
- Can be easily applied in boar studs as the concentration established on the day after production (D1) is relevant for both semen-quality and shelf-life assessments.
- The Pig 4MID[®] approach is today the only approach on the market to assess rigorously **boar fertility** with a molecular marker called proAKAP4
- The Pig 4MID[®] approach evaluates **boar fertility** independently of sow fertility.
- **Easy to implement**: everything is included in each Pig 4MID[®] Kit box, cost of equipment (reader, pipette, etc.) is under 8,000 € for lab set up, a small table or bench space is enough. The 4MID[®] approach does not required highly trained operators (half day of formation is enough).
- **FTE** : The Pig 4MID[®] assay is performed in **2,5 to 3 hours**. However this is not full-time job. The total time spent by person for the use of the Pig 4MID[®] Kit :
 - For one sample : around 27 minutes
 - A complete plate of Pig 4MID[®] Kit of 88 samples : around 67 minutes (with steps of 2' + 30' + 30' + 5')